World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Evaluation of anti diabetic activity of soybean seeds in albino rats

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Received: 01-08-2019 / Revised Accepted: 29-08-2019 / Published: 31-08-2019

ABSTRACT

Diabetes is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period of time. Diabetes is due to either the pancreas doesn't produce any insulin or not enough insulin to help glucose to enter our body's cells – or the insulin that is produced does not work properly. Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of 200 mg/dl were selected for the study. Treatment with soybean seeds was started after 48 hr. alloxan injection. Seven days treated albino Wister rats with highest dose of soaked soybean (500ml/kg), germinated soybean (500mg/kg), soybean seeds powder (500mg/kg) and ethanolic extract of soybean seeds (500mg/kg) significantly lowered elevated blood glucose level as compared to diabetic control rats. This study indicates that the soybean extracts possess significant anti diabetic activity against alloxan induced diabetes. This may be attributed due to presence of phytoconstituents like saponin and isoflavonoids.

Keywords: Diabetes, Alloxan, Ethanolic, Insulin, Soybean

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How to Cite this Article: R. S. Jadhao, V. J. Chawre, K.R. Biyani. Evaluation of anti-diabetic activity of soybean seeds in albino rats. World J Pharm Sci 2019; 7(9): 114-123.

INTRODUCTION

The word diabetes comes from Latin diabetes, which in turn comes from Ancient Greek (diabenin) which literally means "a passer through; a siphon". Indian physicians identified the Disease and classified it as *madhumeha*or "*honey urine*", noting the urine would attract ants. The disorder is also known as '*Prameha*', which means watering. In relation to human disease it may have a meaning of passing urine, qualified by prefix 'pra' meaning excess in frequency and quantity and 'meha' meaning urination.[1,2]

Diabetes is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period of time. Diabetes is due to either the pancreas doesn't produce any insulin or not enough insulin to help glucose to enter our body's cells – or the insulin that is produced does not work properly (known as insulin resistance).[3,4]

Insulin is the hormone produced by the pancreas that allows glucose to enter the body's cells, where it is used as fuel for energy so we can work, play and generally live our lives. It is vital for life. Glucose comes from digesting carbohydrate and is also produced by the liver.[5,6]

Soybean protein administration reduced cholesterol; triglyceride, and Low density lipoprotein levels in healthy persons as well as in diabetic patients, similar effect was also noticed in rats. Soybean is an extremely rich source of protein and fat, and a good source of energy, vitamins and minerals and it has been shown to be hypocholesterolemic in animals.

Soybean is not only protein rich, but also a good source of minerals like phosphorous, calcium, iron and soluble fibre. Soybean proteins complement cereal proteins to provide source of dietary protein of vegetable origin for human beings.

Soybean diet is a good option in type 2 diabetes individuals due toits effect on hypertension, hypercholesterolemia, atherosclerosis and obesity, which are very common diseases in diabetic patients. Furthermore, substituting animal protein for soybean or other vegetable protein may also decrease renal hyper filtration, proteinuria, and renal acid load and therefore reduces the risk of renal disease in type-2 diabetes.

MATERIALS AND METHODS

Collection and authentication of the seeds: The seed of the plant *Glycine Max* were collected from the local market of Buldhana. They were authenticated by Head of Department of botany Shri. Shivaji Science College Chikhli, Maharashtra.

Induction of diabetes in experimental animals: Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of 200 mg/dl were selected for the study. Treatment with soybean seeds was started after 48 hr alloxan injection.

Soaked soybean: Soybean seeds were socked for 8 to 12 hours, socked seeds were ground to a fine paste, and milk was prepared from this past by adding water into it, finally this milk was heated up till boiling. This boiled milk after getting cooled was filtered. This filtered milk was the final form of drug used. All animals were divided in five groups each group contained six animals. First group was treated with vehicle and group second was treated with diabetic control, group third was treated with 100 ml/kg of soybean milk, group forth was treated with 200 ml/kg and group fifth was treated with 500 ml/kg of milk.

Germinated soybean: Soybean seed were soaked for 8 to 12 hours, left for 24 hours for germination at room temperature, germinated soybean seeds were dried. These dried seed were ground into a powder and was used as drug. The solution was prepared with normal saline solution [0.9 gm. NaCl in 100ml water]. All animals were divided in five groups each group contained six animals. First group was treated with vehicle and group second was treated diabetic control, group third was treated with 100 mg/kg of germinated soybean, group forth was treated with 200 mg/kg and group fifth was treated with 500 mg/kg of germinated soybean.

Soybean powder: Dried soybean seed were collected from market these dried seed were ground into a powder and was used as drug. The solution was prepared with normal saline solution [0.9 gm. NaCl in 100 ml water] . All animals were divided in five groups each group contain six animals. First group was treated with vehicle and group second was diabetic control, group third was treated with 100 mg/kg of soybean seed, group forth was treated with 200 mg/kg and group fifth was treated with 500 mg/kg of soybean seed.

Soybean extract: The dried seed powder of *Glycine Max* was extracted at room temperature with the absolute ethanol overnight for the preparation of the extracts for this study. The obtained extract was then concentrated at 50^0 Cuntil a yellow brown

coloured solid mass was obtained further the after drying a yellow brown powder of drug is used for study. The solution was prepared with normal saline solution [0.9 gm. NaCl in 100 ml water]. All animals were divided in five groups each group contain six animals. First group was treated with vehicle and group second was diabetic control, group third was treated with 100 mg/kg of soybean extract, group forth was treated with 200 mg/kg and group fifth was treated with 500 mg/kg of soybean extract.

Collection of blood sample and blood glucose determination: Blood samples were withdrawn from tail tip of rat. Blood glucose estimation a were done on day 1,3,5 and 7 of the study. Blood glucose estimation was done by one touch electronic glucometer using glucose test strips.

Statistical analysis: All the values fasting blood sugar, were expressed as mean ± standard deviation (SD) and analysed for ANOVA and post hoc Dunnet's -test. Differences between groups were considered significant at $P < 0.01$ levels.

RESULT

Phytochemical screening: The Phytochemical screening of Ethanolic extract of leaves of *Glycine Max* revealed the presence of flavonoids, proteins, peptides, linoleic acid, carbohydrates, oil and saponins. Tests for steroids, alkaloids, anthraquinones, glycosides, tannins, lactones, esters, and amino acids were found to be negative.

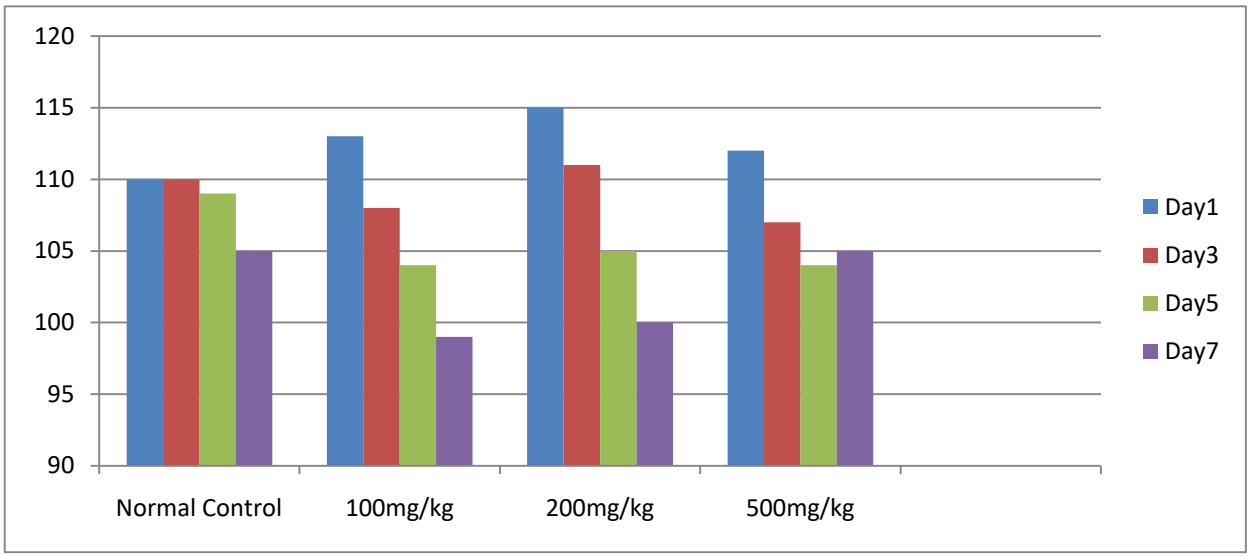
Evaluation of Hypoglycaemic activity of soybean seed

Effect of soaked soybean on blood glucose levels: 7 days treatment of Soaked Soybean at the dose of 100ml/kg, 200ml/kg, and 500ml/kg showed no significant decrease in blood glucose level on days 5 and 7 as compared to normal control group. The result was found to be dose and time dependent. The result obtained with Soaked Soybean on blood glucose levels are given in table 1 and illustrated in Graph 1.

Table 1- Effect of soaked soybean on blood glucose levels (mg/dl)

Treatment Group	Day 1	Day 3	Day 5	Day 7
Normal	110 ±7.40	110±8.25	109±7.39	105.85±6.39
Soaked soybean 100ml/kg p.o	113±11.69	108±12.05	104±12.54	99±12.05
Soaked soybean 200ml/kg p.o	115±12.86	111±10.94	105±12.05	100±11.63
Soaked soybean 500ml/kg p.o	112±12.12	107±11.90	104±11.44	105±11.74

Values are mean (n=6) ± SD



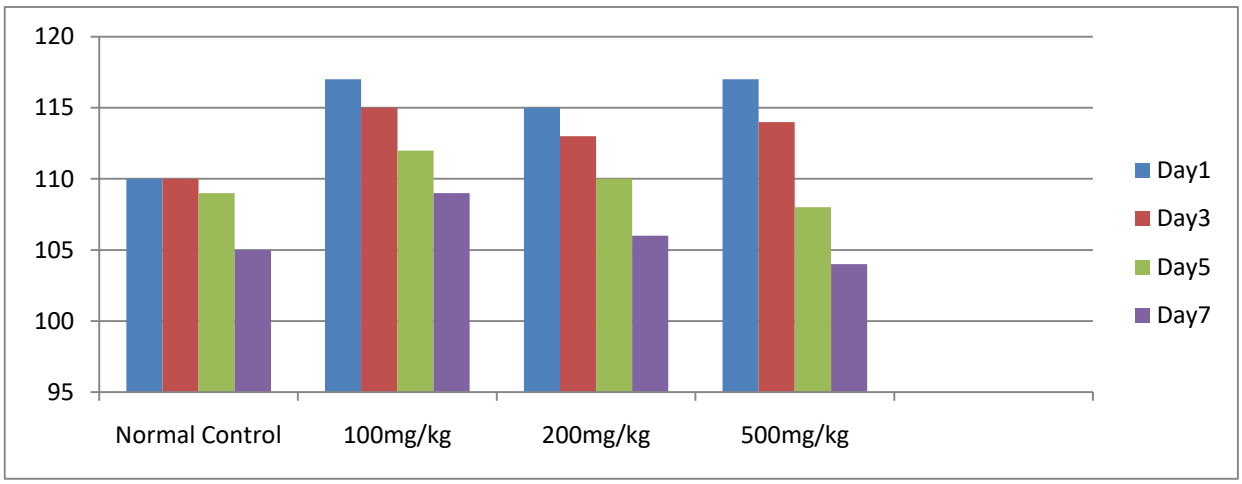
Graph 1- Effect of soaked soybean on blood glucose levels (mg/dl)

Effect of germinated soybean on blood glucose levels: 7 days treatment of Germinated Soybean at the dose of 100mg/kg, 200mg/kg, and 500mg/kg showed no significant decrease in blood glucose level on days 5 and 7 as compaired to normal control group. The result was found to be dose and time dependent. The result obtained with Germinated Soybean on blood glucose levels are given in table 2 and illustrated in Graph 2.

Table 2- effect of germinated soybean on blood glucose levels (mg/dl)

Treatment Group	Day 1	Day 3	Day 5	Day 7
Normal	110±7.40	110±8.25	109±7.39	105±6.39
Germinated soybean 100mg/kg p.o	117±11.39	115±11.05	112±12.44	109±12.14
Germinated soybean 200mg/kg p.o	115±11.84	113±12.94	108±11.04	104±12.33
Germinated soybean 500mg/kg p.o	117±12.12	114±11.90	111±11.44	109±11.74

Values are mean (n=6) ± SD



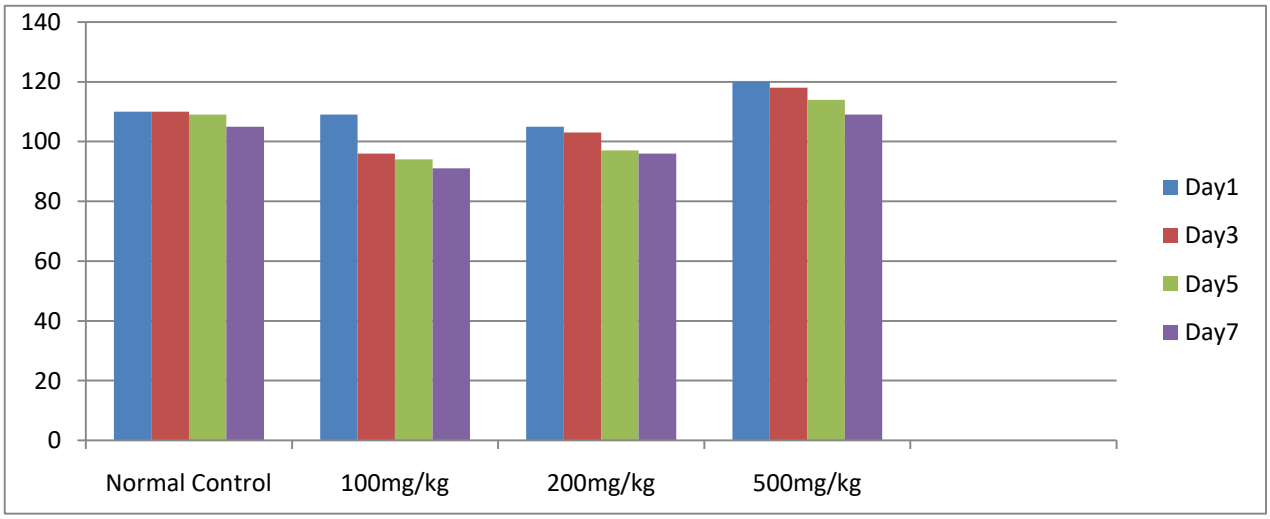
Graph 2- effect of germinated soybean on blood glucose levels (mg/dl)

Effect of powder seeds soybean on blood glucose levels: 7 days treatment of Powder Soybean at the dose of 100mg/kg, 200mg/kg, and 500mg/kg showed no significant decrease in blood glucose level on days 5 and 7 as compared to normal control group. The result was found to be dose and time dependent. The result obtained with powder Soybean on blood glucose levels are given in table 3 and illustrated in Graph 3.

Table 3- effect of powder seeds soybean on blood glucose levels (mg/dl)

Treatment Group	Day 1	Day 3	Day 5	Day 7
Normal	10±7.40	110±8.25	109+_7.39	105±6.39
Powder soybean 100mg/kg p.o	98±13.39	96±12.04	94±11.34	91±10.14
Powder soybean 200mg/kg p.o	105±12.54	103±10.94	97±12.12	96±10.83
Powder soybean 500mg/kg p.o	120±11.12	118±10.90	114±11.66	109±11.70

Values are mean (n=6) ± SD



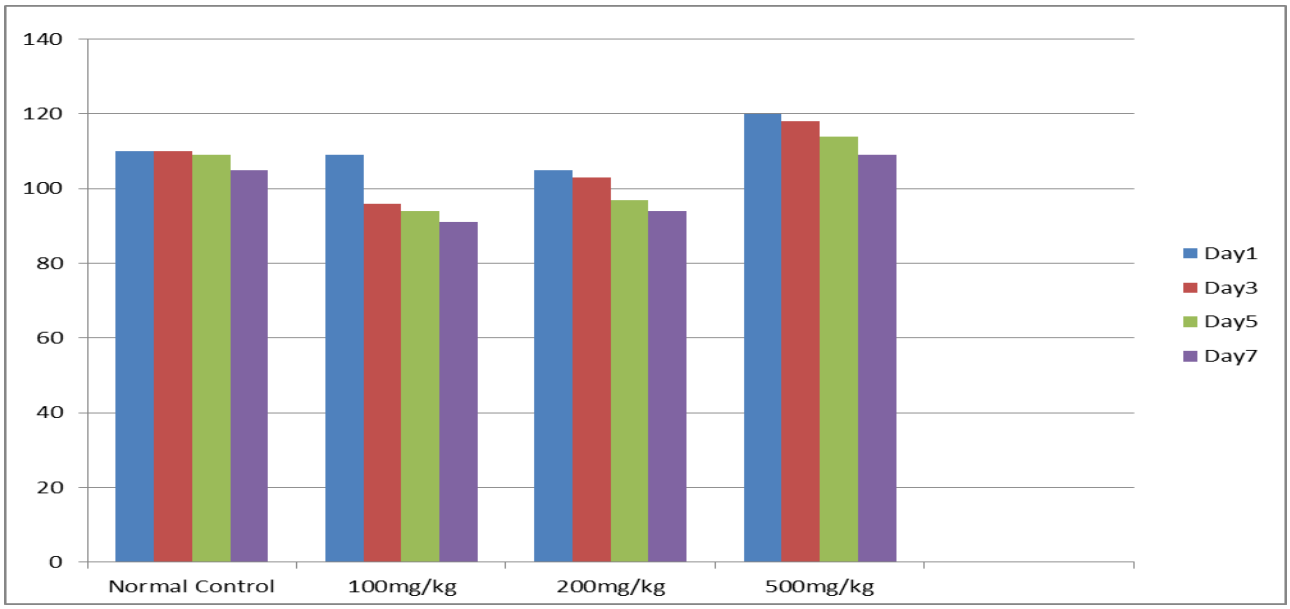
Graph 3- Effect of powder seeds soybean on blood glucose levels (mg/dl)

Effect of soybean extract on blood glucose levels: 7 days treatment of Soybean Extract at the dose of 100mg/kg, 200mg/kg, 500mg/kg showed no significant decrease in blood glucose level on days 5 and 7 as compared to normal control group. The result was found to be dose and time dependent. The result obtained with Soybean extract on blood glucose levels are given in table 4 and illustrated in Graph 4.

Table 4- effect of soybean extract on blood glucose levels

Treatment Group	Day 1	Day 3	Day 5	Day 7
Normal	110±7.40	110±8.25	109±7.39	105±6.39
soybean Extract 100mg/kg p.o	109±11.29	107±11.14	108±12.14	107±11.24
soybean Extract 200mg/kg p.o	104±10.34	102±13.04	102±11.10	101±12.83
soybean Extract 500mg/kg p.o	112±12.22	110±11.80	108±2.56	106±10.50

Values are mean (n=6) ± SD



Graph 4- effect of soybean extract on blood glucose levels

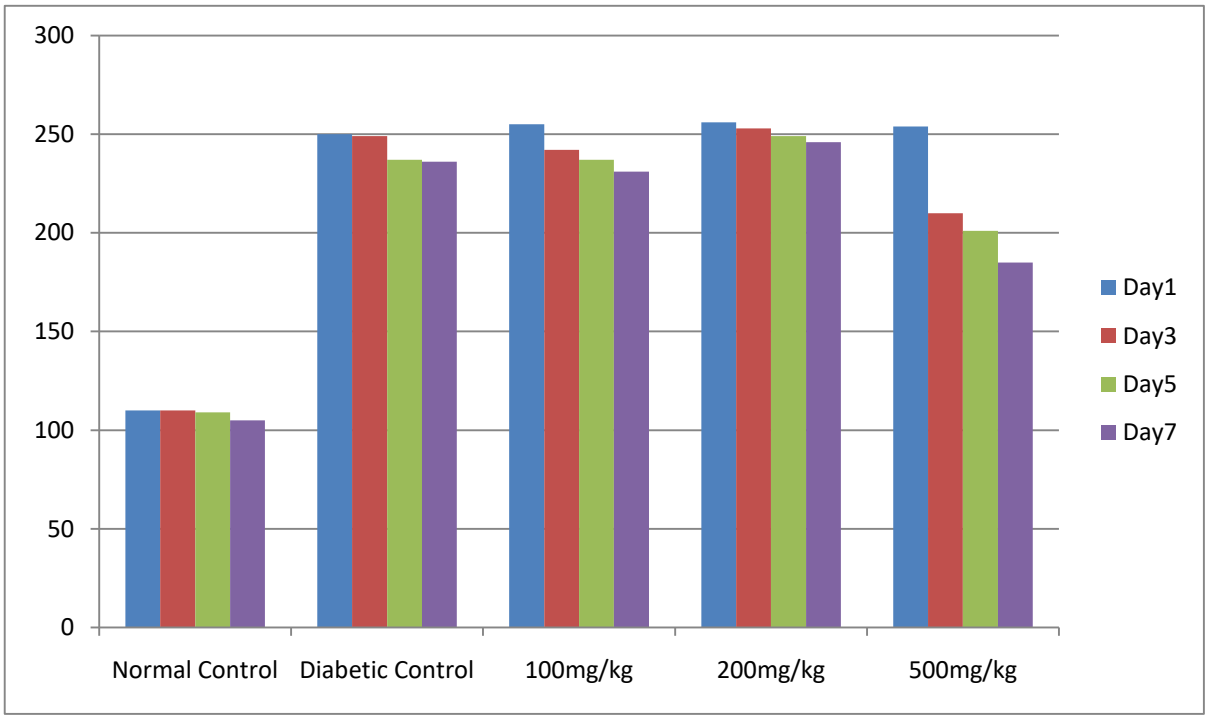
Evaluation of Antidibetic activity of soybean seed in alloxan induce diabetic rat

Effect of soaked soybean on blood glucose levels: 7 days treatment of Soaked Soybean at the dose of 100ml/kg and 200ml/kg, showed no significant decrease in blood glucose level on days 3, 5 and 7 as compared to diabetic control group. 7 days treatment of Soaked Soybean at the dose of 500ml/kg showed significant decrease in blood glucose level on days 3, 5 and 7 as compared to diabetic control group.The result was found to be time dependent. The result obtained with Soaked Soybean on blood glucose levels are given in table 5 and illustrated in Graph 5.

Table 5- effect of soaked soybean on blood glucose levels (mg/dl)

Treatment Group	Day 1	Day 3	Day 5	Day 7
Normal	110±7.40	110±8.25	109±7.39	105±6.39
Diabetic Control	250±8.21	249±9.74	237±12.81	236±6.84
Soaked soybean 100ml/kg p.o	255±11.09	242±12.08	237±12.34	231±12.43
Soaked soybean 200ml/kg p.o	256±11.86	253±12.94	249±13.05	246±14.63
Soaked soybean 500ml/kg p.o	254±13.32	210±12.92	201±13.43	185±13.74

Values are mean (n=6) ± SD



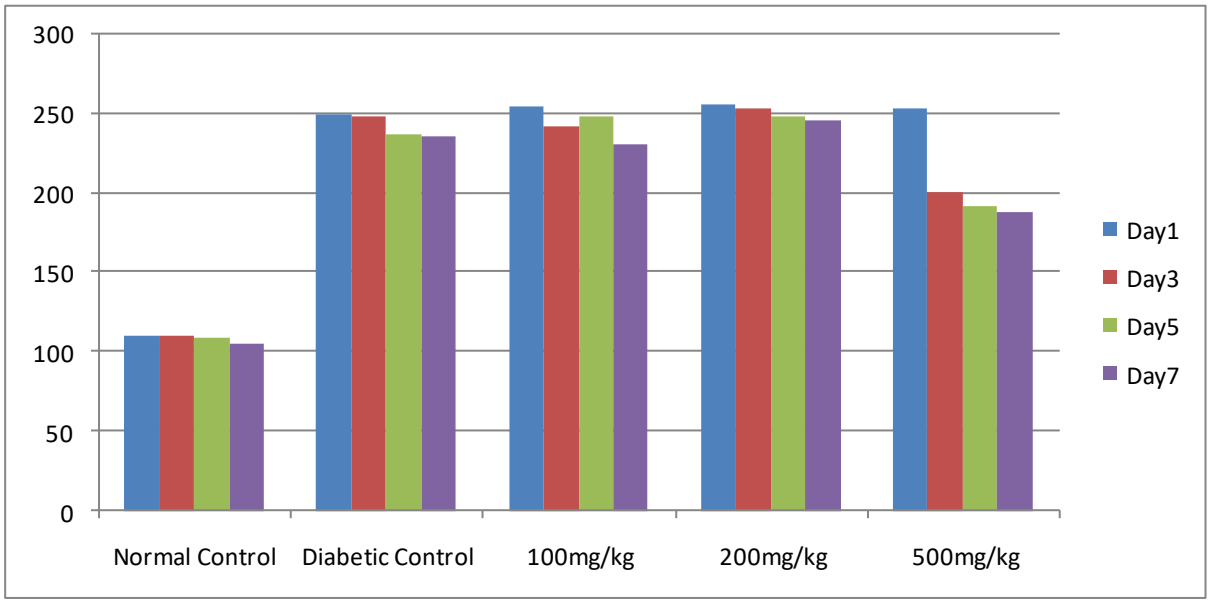
Graph 5- effect of soaked soybean on blood glucose levels (mg/dl)

Effect of germinated soybean on blood glucose levels: 7 days treatment of Germinated Soybean at the dose of 100mg/kg and 200mg/kg showed no significant decrease in blood glucose level on days 3, 5 and 7 as compared to diabetic control group. 7 days treatment of Germinated Soybean at the dose of 500mg/kg showed significant decrease in blood glucose level on days 3, 5 and 7 as compared to diabetic control group. The result was found to be time dependent. The result obtained with Germinated Soybean on blood glucose levels are given in table 6 and illustrated in Graph 6.

Table 6 - effect of germinated soybean on blood glucose levels (mg/dl)

Treatment Group	Day 1	Day 3	Day 5	Day 7
Normal	110±7.40	110±8.25	109±7.39	105±6.39
Diabetic Control	250±8.21	249±9.74	237±12.81	236±6.84
Germinated soybean 100mg/kg p.o	265±11.37	252±12.05	248±12.34	235±13.14
Germinated soybean 200mg/kg p.o	253±11.55	242±12.76	232±11.54	228±12.87
Germinated soybean 500mg/kg p.o	264±12.45	201±11.56	192±11.49	188±11.87

Values are mean (n=6) ± SD



Graph 6 - effect of germinated soybean on blood glucose levels (mg/dl)

Effect of powder soybean on blood glucose levels: 7 days treatment of Powder Soybean at the dose of 100mg/kg and 200mg/kg showed no significant decrease in blood glucose level on days 3, 5 and 7 as compared to diabetic control group. 7 days treatment of Powder Soybean at the dose of 500mg/kg showed significant decrease in blood glucose level on days 3, 5 and 7 as compared to diabetic control group .The result was found to be time dependent. The result obtained with powder Soybean on blood glucose levels are given in table 7 and illustrated in Graph 7.

Table 7- effect of powder soybean on blood glucose levels (mg/dl)

Treatment Group	Day 1	Day 3	Day 5	Day 7
Normal	110±7.40	110±8.25	109±7.39	105±6.39
Diabetic Control	250±8.21	249±9.74	237±12.81	236±6.84
Powder soybean 100mg/kg p.o	255+_2.39	242+_2.98	232+_3.34	227+_3.54
Powder soybean 200mg/kg p.o	253±2.59	243±2.94	239±3.12	225±4.83
Powder soybean 500mg/kg p.o	267±1.56	205±0.96	193±1.99	181±2.98

Values are mean (n=6) ± SD

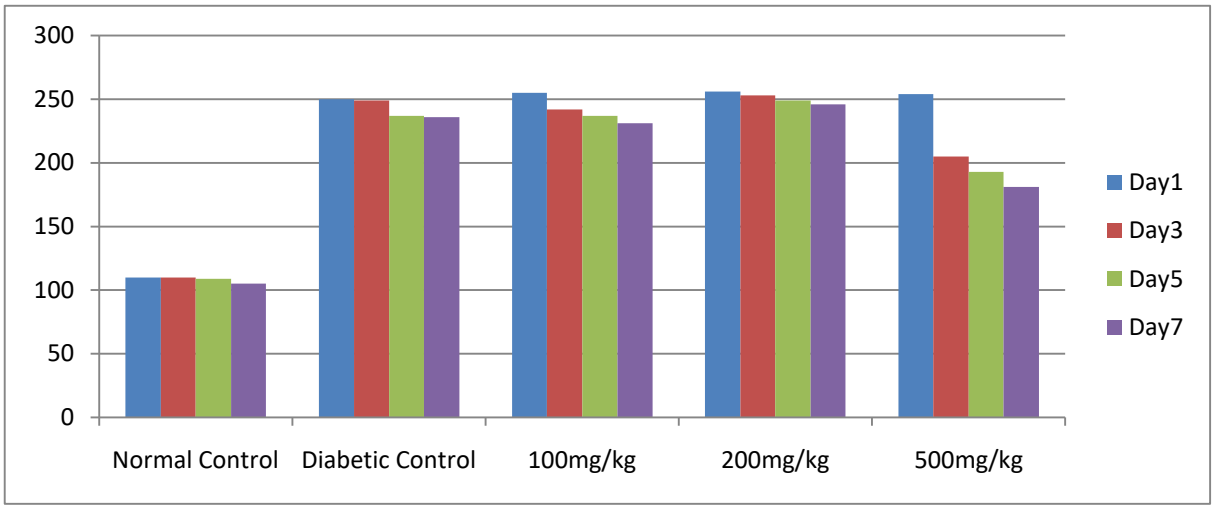


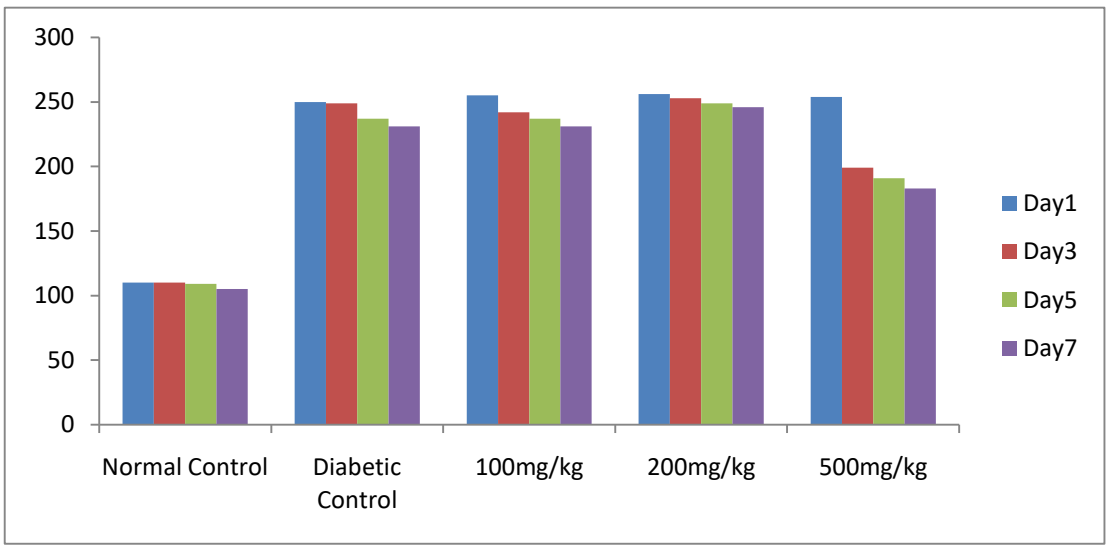
Table 7- effect of powder soybean on blood glucose levels (mg/dl)

Effect of soybean extract on blood glucose levels: 7 days treatment of Soybean Extract at the dose of 100mg/kg and 200mg/kg showed no significant decrease in blood glucose level on days 3, 5 and 7 as compared to diabetic control group. 7 days treatment of Soybean Extract at the dose of 500mg/kg showed significant decrease in blood glucose level on days 3, 5 and 7 as compared to diabetic control group. The result was found to be time dependent. The result obtained with Soybean extract on blood glucose levels are given in table 8 and illustrated in Graph 8.

Table 8- Effect of Soybean Extract on Blood Glucose Levels

Treatment Group	Day 1	Day 3	Day 5	Day 7
Normal	110±7.40	110±8.25	109±7.39	105±6.39
Diabetic Control	250±8.21	249±9.74	237±12.81	236±6.84
soybean Extract 100mg/kg p.o	254±1.29	244±1.14	230±2.14	225±1.24
soybean Extract 200mg/kg p.o	267±0.34	255±3.04	241±1.10	229±2.83
soybean Extract 500mg/kg p.o	244±2.22	199±1.80	191±2.56	183±0.50

Values are mean (n=6) ± SD



Graph 8- Effect of Soybean Extract on Blood Glucose Levels.

DISCUSSION

Since antiquity, diabetes has been treated with plant medicines. A number of experimental and clinical studies have shown the efficacy of various herbs in lowering blood glucose in diabetes. These herbal preparations exhibit their beneficial effects by different mechanisms which may or may not affect insulin release. In view of traditional use of Soybean seedin treating diabetes, the present study was carried out to prove the traditional claims scientifically. Currently, different kinds of synthetic drugs viz. biguanides, diphenylalanine derivatives, glucosidase inhibitors, meglitinides, sulphonylureas, and thiazolidinedione in addition to insulin, are widely used in the management of diabetes all over the world. However, due to untoward side effects, the efficacies of these drugs are quite controversial and there is a strong demand for new but safe drugs for the treatment of diabetes efficaciously.

Plants have been suggested as a rich, as yet unexplored source of potentially useful antidiabetic drugs. However,only a few have been subjected to detailed scientific exploration due to a lack of mechanism based available *in vitro* assays. A number of experiments have shown the beneficial effects of medicinal plants in the management of diabetes mellitus. Numerous mechanisms of actions have been proposed for these plant extracts. Some reports have linked their effects to the activity of pancreatic cells (synthesis, release, cell regeneration/revitalization) or the increase in the inhibitory effect against insulinase and the increase of the insulin sensitivity or the insulin-like activity of the plant extracts. Others have suggested that the mechanisms may involve improved glucose homeostasis, increase of peripheral utilization of glucose, increase of synthesis of hepatic glycogen and/or decrease of glycogenolysis acting on enzymes inhibition of intestinal glucose absorption, reduction of glycaemic index of carbohydrates and reduction of the effect of glutathione.

Alloxan acts as a cytotoxic for beta-cells of the islet of Langerhans, causes diabetes by inducing cell necrosis The Reactive Oxygen Species mediates the cytotoxic action with the increase in cytosolic calcium concentration, leading to rapid beta-cells destruction. This results into decreased insulin secretion and elevated blood glucose level. This experimental study showed that normal nondiabetic albino wister rats received soaked soybean milk, (100ml/kg, 200ml/kg, 500ml/kg), germinated soybean (100mg/kg, 200mg/kg, 500mg/kg), soybean powder (100mg/kg, 200mg/kg, 500mg/kg), soybean extract (100mg/kg, 200mg/kg, 500mg/kg) for 7 days showed no significant decrease in normal blood glucose level as compare to normal control animal. In this study, alloxan at the dose of 120mg/kg was injected intraperitonially. After 48hrs injection of alloxan, blood was withdrawn from tip of tail for estimation of blood glucose level. Diabetic animals having blood glucose level 200mg/dl were selected for study.

This experimental study reveals that alloxan-treated rats received soaked soybean at the dose of 100ml/kg and 200ml/kg showed no significant lower elevated blood glucose level but at the dose of 500ml/kg significantly lowered elevated blood glucose level as compared to the diabetic control group. This experimental study reveals that alloxan-treated rats received germinated soybean at the dose of 100mg/kg and 200mg/kg showed no significant lower elevated blood glucose level but at the dose of 500mg/kg significantly lowered elevated blood glucose level as compared to the diabetic control group. This experimental study reveals that alloxan-treated rats received soybean seeds powder at the dose of 100mg/kg and 200mg/kg showed no significant lower elevated blood glucose level but at the dose of 500mg/kg significantly lowered elevated blood glucose level as compared to the diabetic control group.

This experimental study reveals that alloxan-treated rats received soybean seeds extract at the dose of 100mg/kg and 200mg/kg showed no significant lower elevated blood glucose level but at the dose of 500mg/kg significantly lowered elevated blood glucose level as compared to the diabetic control group may be due to the possibility that few of beta cells are still surviving and stimulated by extract component (s), releasing insulin. In conclusion, this study indicates that the soybean extracts possess significant antidiabetic activity against alloxan induced diabetes. This may be attributed due to presence of phytoconstituents like saponin and isoflevonoids.

CONCLUSION

Seven days treated albino wister rats with soaked soybean (100ml/kg, 200ml/kg, 500ml/kg,), germinated soybean (100mg/kg, 200mg/kg, 500mg/kg), soybean seeds powder (100mg/kg, 200mg/kg, 500mg/kg) and ethanolic extract of soybean seeds (100mg/kg, 200mg/kg, 500mg/kg) did not significantly lower normal blood glucose level as compared to nondiabetic control animals. Alloxan at the dose of 150 mg/kg could significantly elevate blood glucose level in all groups of animals as compared to normal control animals. Seven days treated albino Wister rats with highest dose of soaked soybean (500ml/kg), germinated soybean (500mg/kg), soybean seeds

powder (500mg/kg) and ethanolic extract of soybean seeds (500mg/kg) significantly lowered elevated blood glucose level as compared to diabetic control rats. It is concluded that all forms of soybean seeds at highest dose(500mg/kg) possess antidiabetic activity. This may be due to presence of phytoconstituents saponin and isoflavones.

ACKNOWLEDGEMENT

The author is sincerely thankful to the Dr. K. R. Biyani for rendering his suggestions and helping in each and every step of completing this research paper successfully. Also thankful to MrV. J. Chawre.

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