



**COMPARISON OF THE HYPOGLYCEMIC EFFECT OF FLAX SEEDS
AND METFORMIN IN STREPTOZOTOCIN INDUCED
DIABETIC RATS**

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ABSTRACT

BACKGROUND & OBJECTIVES: Diabetes is characterized by hyperglycemia. Flax seeds are rich in polyunsaturated fatty acids (PUFA's). Diet rich in PUFAs have an important role in enhancing insulin sensitivity & decreasing blood glucose in diabetic rats. Prior studies have been done by adding flax seeds in diet. To ensure administration, the rats were administered flax seed powder suspension

orally by a syringe in the present study. The objective was to compare the hypoglycemic effect of flax seeds with metformin in streptozotocin induced diabetic rats.

METHODOLOGY: The study was initiated after approval from the Institutional animal ethics committee. Male albino-Wistar rats were divided into 5 groups with 5 animals in each group. Normal control, diabetic control, 10% flax seed powder, 20% flax seed powder & metformin. Streptozotocin 30mg/kg was administered by intraperitoneal route to induce diabetes. Baseline fasting blood glucose and body weight was assessed at the commencement of the study and subsequently after 2, 4 and 14 days. **RESULTS:** Statistical analysis was done using one way ANOVA. 10% and 20 % flax seeds reduced fasting blood sugar significantly ($p < 0.05$) when compared to metformin. Among flax seeds, 20% was better in reducing blood sugar significantly ($p=0.001$). In the 20% flax seed group there was more significant reduction in body weight ($p=0.00$). **CONCLUSION:** Based on the results, 20%

flax seeds reduce blood sugar significantly compared with metformin and can be used as a new option in glycemic control in diabetics as an alternative to oral hypoglycemic drugs.

KEYWORDS: Diabetic rats, streptozotocin, metformin, flax seeds, fasting blood sugar.

INTRODUCTION

Diabetes and its complications constitute a major health problem. Diabetes is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.^[1] It is associated with significant morbidity due to specific diabetes related microvascular and macrovascular complications. Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030.^[2] International Diabetes Federation (IDF) has identified DM as the fourth leading cause of human morbidity at 6% (IDF 2007) while its prevalence is much higher in developed countries though its greatest impact will be felt in developing countries (Hossain et al. 2007). Given the high cost of medication, practitioners in India are now looking to control DM with alternatives.^[3]

Flaxseed is the richest source of the lignan secoisolariciresinol diglucoside (SDG). After ingestion, SDG is converted to secoisolariciresinol, which is further metabolised to the mammalian lignans enterodiol and enterolactone. SDG metabolites may protect against cardiovascular disease (CVD) and the metabolic syndrome by reducing lipid and glucose concentrations.^[4] Flax seeds are rich in polyunsaturated fatty acids (PUFAs). Diet rich in PUFAs are very effective in improving cell membrane lipid structure. Especially fatty acids & phospholipids have an important role in enhancing insulin sensitivity & decreasing blood glucose in diabetic rats.^[5] Thus membranes enriched in unsaturated fatty acids tend to bind more insulin than membranes enriched in saturated fatty acids.^[6] Results from previous studies suggest that flax seed has hypoglycemic effect in streptozocin induced diabetes in rats.^[7]

Metformin is the standard oral hypoglycemic drug. Metformin decreases hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis).^[8] It has been demonstrated that it has a novel mechanism by which it reduces plasma glucose level, which is a noninsulin dependent hypoglycemic action.^[9] The deficient functions of pancreatic beta cells in STZ-induced diabetic rats have been documented.^[10] Rats with STZ-induced diabetes would thus be used in the current study as an animal model of type 1-like diabetes.

Though prior studies,^[7] have been done by mixing flax seed powder with the standard diet (pellet form), to ensure administration, the rats would be administered flax seed powder suspension orally by a syringe in the present study. So this study aims to compare the hypoglycemic effect of flax seed powder with metformin.

OBJECTIVES

Primary aim

To compare the hypoglycemic effect of flax seeds with metformin in streptozotocin induced diabetic rats.

Secondary aim

To assess the hypoglycemic effect of 10% and 20% flax seed powder in streptozotocin induced diabetic rats.

To assess the effect of flax seeds on body weight of rats.

METHODOLOGY

Animals

30 Male albino-Wistar rats weighing 180-300g were used in the present study. All the rats were kept at room temperature of 22-25°C in the animal house. The internationally accepted ethical guidelines for the care of laboratory animals were followed. Prior to the experiments, rats were fed with standard food for 1 week in order to adapt to the laboratory conditions. All animal procedures were performed after approval **from the institutional animal ethics committee** and in accordance with the recommendations for the proper care and use of laboratory animals.

MATERIALS USED

Streptozotocin – 30mg/kg,^[11] Metformin – 200 mg/kg, Brown flax seeds – 10% & 20%. Streptozotocin was purchased, preserved at 25°C and used for this study. Metformin was purchased from the market and stored at room temperature. Brown flax seeds were purchased commercially from the market and powdered. 10 % and 20% flax seed powder suspensions were prepared. Both metformin and flax seed powder were administered orally to the rats as a suspension by a 1ml syringe (water was used as the vehicle).

Induction of diabetes mellitus

Streptozotocin (STZ) (30mg/kg)¹² was dissolved in 50 mM sodium citrate(PH 4.5) solution containing 150 mM NaCl. The solution was administered by intraperitoneal route

immediately after preparing the solution. Animals were fed with 5% Dextrose in order to prevent hypoglycemic shock for 18 hours,^[13] Fasting blood sugar was estimated after 4 days to confirm the development of diabetes mellitus.

Estimation of blood sugar

Baseline blood sugar was assessed in all the rats after fasting for 12 hours before start of the experiment by glucometer (a drop of blood was taken from the tail vein by making a small incision). Then it was assessed after 4 days of inj Streptozotocin to confirm the development of diabetes in the animals. The rats which had a fasting blood glucose value of more than 170mg/dl were considered diabetic and used in the study. After that blood sugar was assessed after 48 hours, 96 hours and 14 days.

Experimental design

25 male Wistar rats were used in this study with 5 rats in each of the following groups

Group 1 (normal control) - received only the vehicle orally by syringe.

Group 2 (diabetic control) – received only the vehicle orally by syringe.

Group 3 (10% flax seed powder) - Diabetic rats received 10% flax seed powder as suspension orally by syringe.

Group 4 (20% flax seed powder group) - Diabetic rats received 20% flax seed powder in suspension orally by syringe.

Group 5 (Metformin group) – Diabetic rats received 200mg/kg metformin as a suspension orally by syringe.

Following parameters were assessed

Fasting blood glucose

Blood sample was collected from the tail vein of the rat and fasting blood glucose was assessed by glucometer. Baseline blood sugar was assessed in all the rats before start of the experiment by glucometer. Then it was assessed after 4 days of inj. Streptozotocin to confirm the development of diabetes in the animals. After that blood sugar was assessed after 48 hours, 96 hours and 14 days.

Body weight

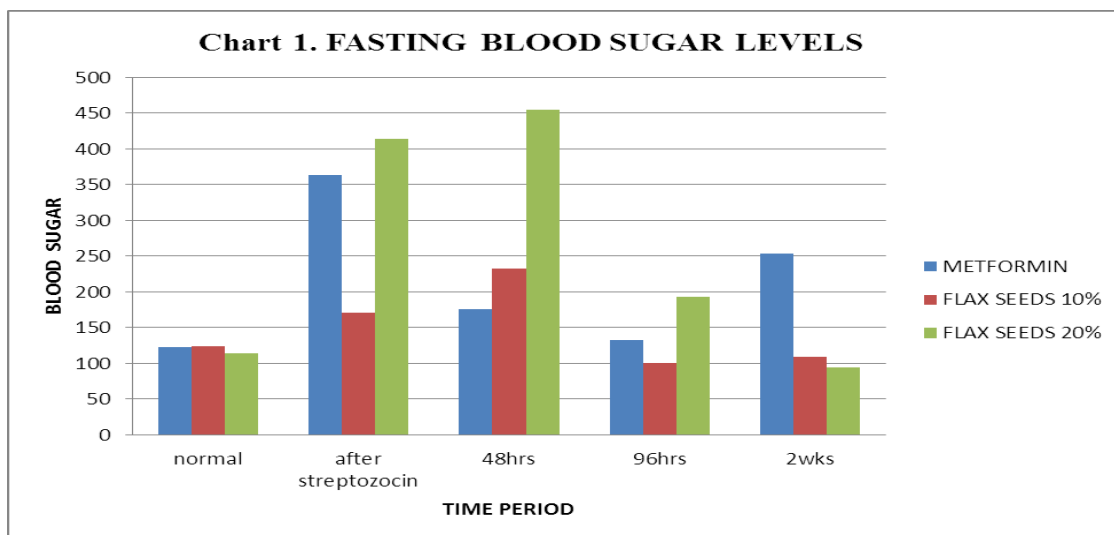
Body weight was assessed at baseline and after 48 hours, 96 hours and 14 days.

Statistical analysis

The data were expressed as mean \pm SD, and statistical analysis was carried out by one-way analysis of variance (ANOVA). Differences were considered significant if the *P* value was <0.05 .

RESULTS

After confirmation of diabetes in the rats, fasting blood sugar and body weight were assessed after 48 hours, 96 hours and 14 days. The fasting blood sugar after 14 days in the metformin group decreased from 364 ± 147 mg/dl to 253 ± 208 mg/dl ($p=0.36$), while in the flax seed 10% group it decreased from 171 ± 29 mg/dl to 108 ± 34 mg/dl ($p=0.016$) and in the flax seed 20% group it decreased from 413 ± 143 mg/dl to 94 ± 20 mg/dl ($p=0.001$). Amongst the three groups the reduction in fasting blood sugar in the 10% and 20% groups flax seeds was statistically significant ($p < 0.05$).



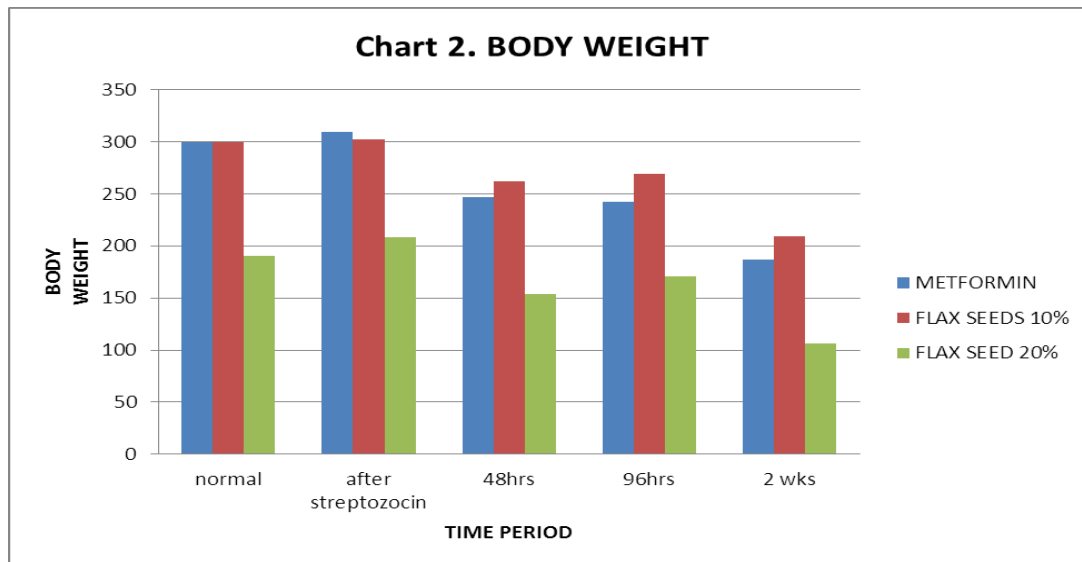
The fasting blood sugar after 48 hours in the metformin, flax seeds 10% and 20% groups were 176 ± 82 mg/dl, 232 ± 111 mg/dl and 455 ± 46 mg/dl respectively. Metformin showed significant fall in blood glucose after 48 hours when compared to 10% and 20% flax seeds.

After 96 hours the fasting blood sugar in the metformin, 10% and 20% flax seeds groups were 132 ± 43 mg/dl, 100 ± 7 mg/dl and 193 ± 98 mg/dl respectively. 10% flax seeds showed significant fall in blood glucose after 96 hours when compared to metformin and 20% flax seeds.

Table 1. Differences between Metformin, 10% & 20% Flax seed groups expressed as Mean \pm SD

METFORMIN		
	BLOOD SUGAR	BODY WEIGHT
TIME PERIOD	MEAN\pm SD	MEAN\pm SD
NORMAL	123 \pm 7.7	300 \pm 81
AFTER STREPTOZOCIN	364\pm147	309\pm70
AFTER 48 HRS	176 \pm 82	247 \pm 65
AFTER 96 HRS	132 \pm 43	242 \pm 76
AFTER 2 WEEKS	253\pm208	187\pm64
FLAX SEEDS 10%		
	BLOOD SUGAR	BODY WEIGHT
TIME PERIOD	MEAN\pm SD	MEAN\pm SD
NORMAL	123 \pm 3	300 \pm 39
AFTER STREPTOZOCIN	171\pm29	302\pm61
AFTER 48 HRS	232 \pm 111	262 \pm 58
AFTER 96 HRS	100 \pm 7	269 \pm 50
AFTER 2 WEEKS	108\pm34	209\pm56
FLAX SEEDS 20%		
	BLOOD SUGAR	BODY WEIGHT
TIME PERIOD	MEAN\pm SD	MEAN\pm SD
NORMAL	123 \pm 3	190 \pm 46
AFTER STREPTOZOCIN	413\pm143	208\pm25
AFTER 48 HRS	455 \pm 46	154 \pm 35
AFTER 96 HRS	193 \pm 98	171 \pm 34
AFTER 2 WEEKS	94\pm20	106\pm28

Body weight measured after 48 hours in metformin, 10% and 20% flax seeds groups was 247 \pm 65 gm, 262 \pm 58 gm and 154 \pm 35 gm respectively. 20% Flax seeds showed greater weight reduction when compared to metformin and 10% flax seeds groups.



After 96hrs there was significant reduction in body weight in the 20% flax seeds group when compared to 10% flax seeds and metformin groups. The body weight measured in metformin, 10% and 20% flax seeds groups was 242 ± 76 gm, 269 ± 50 gm and 171 ± 34 gm respectively.

Body weight of the rats measured after 2 weeks showed that in the metformin group it decreased from 309 ± 70 gm to 187 ± 64 gm ($p=0.02$); while in the 10% flax seed group it decreased from 302 ± 61 gm to 209 ± 56 gm ($p=0.03$) and in the 20% flax seed group it decreased from 208 ± 25 gm to 106 ± 28 gm ($p=0.00$). At the end of 2 weeks, there was significant reduction in body weight in all the three groups. But in the 20% flax seeds group there was more significant reduction.

Table 2: DIFFERENCE BETWEEN GROUPS

BLOOD SUGAR		
TIME PERIOD	BETWEEN GROUP	SIGNIFICANCE
NORMAL	NO SIGNIFICANT DIFFERENCE	NIL
AFTER STREPTOZOCIN	METFORMIN OVER BOTH 10% & 20%	0.008/0.02
AFTER 48 HRS	20% OVER METFORMIN & 10%	0.000/0.001
AFTER 96 HRS	20% OVER 10 %	0.03
AFTER 2 WEEKS	20% OVER METFORMIN	0.05
BODY WEIGHT		
TIME PERIOD	BETWEEN GROUP	SIGNIFICANCE
NORMAL	METFORMIN OVER 20%	0.012
AFTER STREPTOZOCIN	NO SIGNIFICANT DIFFERENCE	NIL
AFTER 48 HRS	NO SIGNIFICANT DIFFERENCE	NIL
AFTER 96 HRS	NO SIGNIFICANT DIFFERENCE	NIL
AFTER 2 WEEKS	20% OVER 10%	0.009

DISCUSSION

Though prior studies.^[7] have been done by mixing flax seed powder with the standard diet (pellet form), to ensure administration, the rats were administered flax seed powder orally by a syringe in the present study.

After confirmation of diabetes in the rats, fasting blood sugar and body weight were assessed after 48 hours, 96 hours and 14 days. As shown in chart 1, the fasting blood sugar after 14 days in the metformin group decreased but it was not statistically significant, while in the flax seed 10% and 20% group fasting blood sugar decreased significantly. As shown in table 1, amongst the three groups the reduction in fasting blood sugar in the 10% and 20% groups flax seeds was statistically significant ($p<0.05$).

Metformin showed significant fall in blood glucose after 48 hours when compared to 10% and 20% flax seeds. As shown in chart 1. Blood sugar lowering by metformin was immediate but not persistent. But 20% flax seeds show gradual and sustained blood sugar lowering and maintenance.

Based on the fasting blood glucose and body weight, the differences between the 3 groups after 48 hours, 96 hours and 14 days is shown in Table 2.

Body weight of the rats measured after 2 weeks showed that in the metformin group, 10% flax seed group and in the 20% flax seed group there was statistically significant weight reduction ($p < 0.05$). But in the 20% flax seeds group there was more significant reduction ($p=0.00$).

Flax seeds are rich in polyunsaturated fatty acids (PUFAs). Diet rich in PUFAs are very effective in improving cell membrane lipid structure. Especially fatty acids & phospholipids have an important role in enhancing insulin sensitivity & decreasing blood glucose in diabetic rats.^[5] Thus membranes enriched in unsaturated fatty acids tend to bind more insulin than membranes enriched in saturated fatty acids.^[6] Results from previous studies suggest that flax seed has hypoglycemic effect in streptozocin induced diabetes in rats.^[7]

In one study they showed antidiabetogenic property of *Linum usitatissimum* active fraction (LU6) in streptozotocin (STZ) induced diabetic Swiss mice. The histochemical and immunohistochemical analysis on pancreatic islets suggested the role of LU6 fraction in islet regeneration and insulin secretion as evident in increase functional pancreatic islets producing insulin.^[14]

Studies using flax seeds in streptozotocin induced diabetic rat models for a longer duration of time is required. Similar studies to understand the mechanism of action behind these effects of flax seeds is necessary. In this study 20% flax seeds showed significant reduction in fasting blood glucose when compared to the standard drug metformin after 2 weeks. 20% flax seeds also showed a significant reduction in body weight.

Given the high cost of medication, practitioners in India are now looking to control DM with alternatives. Flax seeds can be used as an alternative to standard oral hypoglycemic agents for the treatment of diabetes mellitus.

CONCLUSION

Based on the results, 20% flax seeds reduce fasting blood sugar and body weight significantly when compared to metformin in streptozotocin induced diabetic rats. Similar studies to understand the mechanism of action behind these effects of flax seeds is necessary.

Flax seeds are known to have hypolipidemic actions and hence can reduce the complications of diabetes mellitus. Given the high cost of medication, practitioners in India are now looking to control diabetes with alternatives. Flax seeds can be used as an alternative to standard oral hypoglycemic agents for the treatment of diabetes mellitus.

Conflict of interest – None

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REFERENCES

1. El-Atat F, McFarlane SI, Sowers JR: Diabetes, hypertension, and cardiovascular derangements: pathophysiology and management. *Curr Hypertens Rep.*, 2004; 6: 215–223.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care.*, 2004; 27: 1047-1053.
3. McIntosh A, Hutchinson A, Home PD. 2001. Clinical guidelines and evidence review for Type 2 diabetes: management of blood glucose. Sheffield: ScHARR, University of Sheffield.
4. Jennifer L. Adolphe, Susan J. Whiting. Health effects with consumption of the flax lignan secoisolariciresinol diglucoside. *British Journal of Nutrition.*, 2010; 103: 929–938 doi:10.1017/S0007114509992753
5. Zakaria El-Khayat et al, Role of cell membrane fatty acids in insulin sensitivity in diabetic rats treated with flax seed oil. *International journal of pharmacy & pharmaceutical sciences.*, 2013; 5(2).
6. Liu S, Baracos VE, Quinney HA, Clandinin T. Dietary ω -3 and polyunsaturated fatty acids modify fatty acyl composition and insulin binding in skeletal muscle sarcolemma. *Biochemical Journal.*, 1994; 299: 831-837.

7. Maina J K., Kareru P G, Gatebe E G, Rotich H, Githira P N, Njonge F, Kimani Dand Mutembei J K, Hypoglycemic effects of selected herbal drug formulations from the Kenyan market,*J. Nat. Prod. Plant Resour.*, 2014; 4 (4): 10-17
8. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update [PDF]. *Ann Intern Med.*, 2002; 137(1): 25–33. PMID 12093242.
9. Juel-Tan cheng et al, Novel mechanism for plasma glucose lowering action of metformin in streptozocin induced diabetic rats,*Diabetes.*, vol-55, march 2006.
10. Wohaieb SA, Godin DV: Alterations in free radical tissue-defense mechanisms in streptozotocin-induced diabetes in rat: effect of insulin treatment. *Diabetes.*, 1987; 36: 1014 –1018.
11. A. Akbarzadeh, D. Norouziyan, M. R. Mehrabi, et al. Induction of diabetes by Streptozotocin in rats *Indian J Clin Biochem.* 2007 September; 22(2): 60–64. doi: 10.1007/BF02913315 PMID:PMC3453807.
12. Peres GB, Juliano MA, Aguiar JA, Michelacci YM. Streptozotocin-induced diabetes mellitus affects lysosomal enzymes in rat liver. *Braz J Med Biol Res.*, 2014; 47(6): 452-60. E pub 2014 May 9. PMID:24820066
13. Prince PSM et al, Hypoglycemic activity of *Syzygium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. *Journal of Ethnopharmacology*, 1998; 61(1): 1-7.
14. Menakshi Bhat Dusane, Bimba N. Joshi. Beneficial effect of flax seeds in streptozotocin induced diabetic mice: isolation of active fraction having islet regenerative and glucosidase inhibitory properties, *Canadian journal of physiology and pharmacology.*, 2013; 91(5): 325-331.