ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation

Journal Home Page: https://ijrps.com

High Fat Diet and Low Dose Streptozotocin Induced Diabetic Dyslipidaemia and Hepatic Damage in Rats

Sudesh N. Gaidhani*[1], Viswanth Reddy S.[1], Srikanth Ala[1], Sudhakar D.[2], Sanjaya Kumar Y. R.[3]

[1]Department of Pharmacology, National Ayurveda Research Institute Panchakarma, Cheruthuruthy, Thrissur-679 531, Kerala, India
[2]Department of Pharmacology, Central Council for Research in Ayurvedic Sciences, New Delhi, India
[3]National Ayurveda Research Institute for Panchakarma, Cheruthuruthy, Thrissur-679531, Kerala, India

Article History:

Received on: 20 Sep 2022
Revised on: 27 Oct 2022
Accepted on: 29 Oct 2022

Keywords:

HFD,
STZ,
NPD,
Metabolic syndrome,
Diabetic Dyslipidaemia,
Insulin,
Hepatic Cirrhosis

ABSTRACT

The objective of the present study was to validate a rat model of diabetic dyslipidaemia along with hepatic damage by High fat diet and low dose streptozotocin combination that resembles the natural history of metabolic syndrome in humans. Male and Female *Sprague Dawley* rats were divided into 2 groups. One group of rats fed with high fat diet and other group was fed with normal pellet diet for a period of 2 weeks followed by streptozotocin administration on 15^{th} day. Body weight of the animals was measured on day 0, 14 and 21, also post 1 week of the streptozotocin administration the blood glucose, serum lipid, liver, kidney markers and insulin levels were measured. The high fat diet fed animals had exhibited a significant increase in body weight post two weeks of HFD feeding, but the body weight was reduced post 1 week of streptozotocin intervention due to metabolic disruption caused by STZ. The HFD group animals exhibited significant increase in blood glucose, serum total cholesterol, triglycerides, LDL, VLDL. Similarly, a significant decrease in HDL and serum insulin levels were observed as compared to the NPD fed animals. Further the HFD group animals had shown significant elevation of the liver function enzymes such as ALT and ALP levels as compared to the NPD fed animals. The present study confirms the experimental induction of diabetic dyslipidaemia along with hepatic damage which serves as an ideal model for metabolic syndrome along with hepatic cirrhosis which would useful for evaluating the therapeutic agents against type 2 diabetes, hyperlipidaemia and hepatic cirrhosis.

*Corresponding Author

Name: Sudesh N. Gaidhani
Phone: -
Email: sudeshgaidhani@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v13i4.3835

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Diabetes is a chronic progressive disorder which impairs the metabolism of blood glucose. The most common type of diabetes is of Type-2. Type 2 diabetes has strong links with obesity as per the report given by the national institution of diabetes and digestive and kidney diseases. The important risk factors of diabetes are family history, sedentary lifestyle, overweight, high cholesterol and blood pressure [1].

Obesity is related with decreased physical inactivity which increases the risk of diabetes. Studies have reported that increased intake high rich calorie food

may be predisposed to diabetes mellitus. Reduced intake of calories decreases the risk of diabetes and improves the tissue sensitivity of insulin. Regular physical activity lowers the blood sugar levels in obese patients by increasing the uptake of glucose by body cells [2].

The prevalence of diabetes in India has significantly increased in recent years and as per the report of ICMR, it is prevalent in 13% of urban and rural population. Balanced diet and lifestyle changes are essential in the management and most of the people require pharmacological intervention to manage blood sugar levels and its complications [3].

Hence it is necessary to explore wide variety of options for the effective management of diabetes mellitus. The preclinical screening models of type 2 diabetes should reflect the human type 2 diabetes such as insulin resistance/lower insulin secretion instead of total beta cell damage as observed in type 1 diabetes mellitus. Although Non-Obese Type 2 diabetes models such as Goto–Kakizaki (GK) rats and hIAPP mice are employed in diabetes research, they are limited in usage due to their inability to mimic the human pathological scenario. Administration of streptozotocin alone results in pancreatic β-cell destruction that may lead to type-1 DM and also High Fat Diet feeding alone showed increased pancreatic β-cell mass, enhanced insulin secretion, and exocytosis [4]. This can be overcome by administration of low-dose streptozotocin (35mg/kg) after priming the pancreatic beta cells with significant duration of high-fat diet administration cannot produce extensive destruction of beta cell, rather produces mild to moderate hypoinsulinaemia and hyperglycaemia. This stands as an explanatory mechanism behind successful induction of type 2 diabetes with high fat diet – low dose streptozotocin model.

In the current study, the high-fat diet and low dose of streptozotocin was selected as a model for induction of type-2 diabetes mellitus in *Sprague Dawley* rats and also to evaluate the impact of this model on status of lipid and liver markers which resembles the diabetic dyslipidaemia along with hepatic damage.

MATERIALS AND METHODS

Animals

The study was performed in male and female *Sparge Dawley* rats. Animals were procured from the small animal veterinary breeding station, Mannuthy, Thrissur, Kerala. The study was conducted as per the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for Laboratory Animal Facility after approval of Institutional Animal Ethics Committee (IAEC/NARIP/2018-19/02), NARIP, Cheruthuruthy.

Feed and High-Fat Diet

High-fat diet was procured from National Institute of Nutrition, Hyderabad. The composition of high fat diet includes the 58% Fat, 25% Protein and 17% Carbohydrates [5].

One kg of High Fat Diet comprises of following ingredients

1. Powdered NPD:365 g
2. Lard: 310 g
3. Casein: 250 g
4. Cholesterol: 10 g
5. Vitamin and Mineral mix: 60 g
6. D-L-Methionine: 03 g
7. Yeast Powder: 01 g
8. Sodium Chloride: 01 g

Streptozotocin: Streptozotocin was procured from Sigma Aldrich (CAS no. 18883-66-4).

Environment, Housing Acclimatization

One animal was housed in a cage which was designed for Rat. Each cage was identified with cage card, which displayed with study number, cage number, sex, duration of study and animal identification number. Animals were free access to food and water ad. libitum. Animals were acclimatized for 1 week before initiating the study.

Methodology

The study was conducted as per the procedure described by Sreenivasan et. Al. The male and female SD rats were randomized based on body weight. The study design was mentioned in the Table 1. One group of male and female rats serves as high fat diet control which were maintained on high fat diet for a period of 2 weeks. On day 15^{th}, low dose of streptozotocin (35 mg/kg) was injected intraperitoneally to these animals. Similarly, another groups male and female rats were maintained with normal pellet diet for 2 weeks and without streptozotocin intervention on day 15.

The body of the animals of the current study was measured prior to start of the study (day 0), two weeks after feeding of HFD/NPD (14^{th} day) and also post one week of streptozotocin administration.

The blood glucose and other biochemical parameters were measured after two weeks of HFD feeding and post 1 week of streptozotocin administration [5].

Estimation of Insulin

Serum insulin levels were determined post 1 week of streptozotocin administration using ELISA kit (BT LAB, Ct.No. E0707 Ra) as per the manufacturer instructions.

Statistical Analysis

Results obtained from the present study were expressed as Mean ±SEM. Data was subjected to statistical analysis through one way analysis of variance (ANOVA) with multiple comparisons using Sidak's test by using Graph pad prism software. $p<0.05$ was considered as statistically significant.

RESULTS

Glucose

The blood glucose levels of all the groups were checked on day 0 (prior to start of the study) and 21^{st} day (post 1 week of streptozotocin administration). No significant difference in blood glucose levels were observed in high fat diet animals as compared to the normal pellet diet animals on day 0. After streptozotocin intervention the high fat diet fed animals has shown significant increase in the blood glucose levels ($p<0.0001$) [Table 2 and Figure 1].

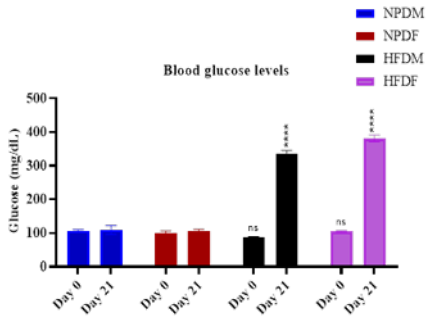

Figure 1: Blood Glucose Levels

Cholesterol, LDL, HDL, VLDL and Triglyceride Levels

The serum Cholesterol, LDL HDL, VLDL & Triglyceride levels of animals were measured on day 0 (prior to start of the study) and 21^{st} day (post 1 week of streptozotocin administration).

No significant difference in cholesterol and other lipid levels were observed in high fat diet animals as compared to the normal pellet diet animals on day 0 ($p>0.05$).

Post streptozotocin administration the high fat diet animals had shown significant ($p<0.05$) ($p<0.01$) increase in the cholesterol levels, LDL levels ($p<0.01$) ($p<0.001$), VLDL($p<0.05$) ($p<0.01$) & Triglyceride ($p<0.05$) ($p<0.01$) levels.

Similarly, a significant decrease in HDL levels were observed in streptozotocin treated HFD animals as compared to the NPD animals($p<0.05$) ($p<0.01$) [Table 3, Figure 2, Figure 3, Figure 4, Figure 5 and Figure 6].

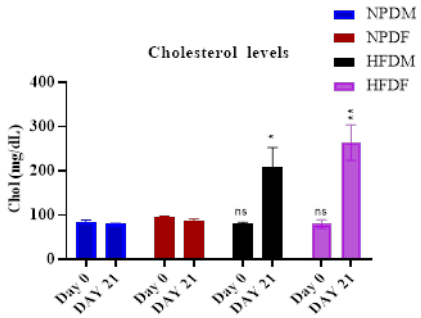

Figure 2: Serum Total Cholesterol Levels

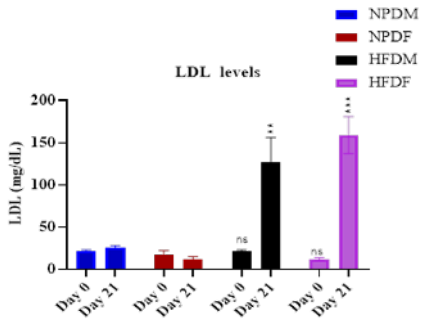

Figure 3: Serum LDL Levels

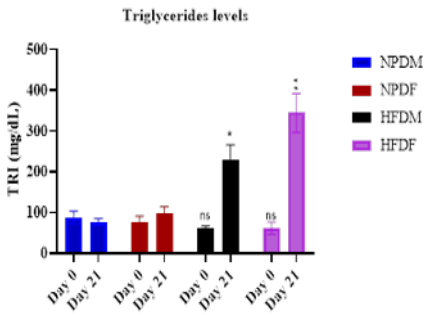

Figure 4: Serum Triglyceride Levels

Liver Markers

The liver enzymes AST, ALT &ALP levels of animals were measured on day 0 (prior to start of the study) and 21^{st} day (post 1 week of streptozotocin administration).

No significant changes in these enzymes' levels were found in high fat diet animals as compared to the

Table 1: Study Design

Group	No of animals	Feed for 2 weeks	STZ intervention
NPDM	03	Normal pellet diet	NO
NPDF	03	Normal pellet diet	NO
HFDM	03	High fat diet	YES
HFDF	03	High fat diet	YES

NPDM- Normal pellet diet male, NPDF- Normal pellet diet Female, HFDM- High fat diet male, HFDF- High fat diet Female, STZ-Streptozotocin

Table 2: Blood Glucose Levels (mg/dl)

Group	Day 0	Day 21
NPDM	107±03	109±13
NPDF	98±08	105±06
HFDM	$86\pm03^{@ns}$	$334\pm11^{@****}$
HFDF	$105\pm02^{\$ns}$	$381\pm10^{\$****}$

NPDM-Normal pellet diet male, NPDF- Normal pellet diet Female, HFDM- High fat diet male, HFDF- High fat diet Female
[Values are expressed as mean ± SEM of 3 rats] ****p<0.0001; ^{ns}-Non significant; Data was subjected to analysis through one-way ANOVA with Sidak's multiple comparison test; The comparison is as follows; @-comparsion of HFDM with NPDM; $- comparison of HFDF with NPDF

Table 3: Serum Cholesterol and Lipid Levels (mg/dl)

Group	Chol (mg/dl)		LDL (mg/dl)		HDL (mg/dl)		VLDL (mg/dl)		TRI (mg/dl)	
	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
NPDM	85±04	80±02	22±01	26±02	46±03	49±01	18±03	15±02	88±15	77±08
NPDF	96±02	88±03	17±05	11±04	64±01	57±04	15±03	20±03	75±16	97±17
HFDM	81±03 $^{@ns}$	209±44 $^{@*}$	21±02 $^{@ns}$	127±29 $^{@**}$	48±03 $^{@ns}$	30±05 $^{@*}$	12±02 $^{@ns}$	46±06 $^{@*}$	60±07 $^{@ns}$	228±38 $^{@*}$
HFDF	80±10 $^{\$ns}$	264±40 $^{\$**}$	12±02 $^{\$ns}$	159±22 $^{\$***}$	55±05 $^{\$ns}$	31±04 $^{\$**}$	12±03 $^{\$ns}$	69±10 $^{\$**}$	62±15 $^{\$ns}$	344±48 $^{\$**}$

NPDM-Normal pellet diet male, NPDF- Normal pellet diet Female, HFDM- High fat diet male, HFDF- High fat diet Female
[Values are expressed as mean ± SEM of 3 rats]*p<0.05, **p<0.01; ***p<0.001; ****p<0.0001;^{ns}- Non significant; Data was subjected to analysis through one way ANOVA with Sidak's multiple comparison test; @-comparison of HFDM with NPDM, $- comparison of HFDF with NPDF

Table 4: Liver Enzyme Levels

Group	AST (U/L)		ALT (U/L)		ALP (U/L)	
	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
NPDM	103±03	164±06	54±04	56±04	228±33	279±78
NPDF	88±06	113±13	46±02	55±07	146±14	267±43
HFDM	$147\pm39^{@ns}$	$162\pm03^{@ns}$	$62\pm09^{@ns}$	$103\pm06^{@**}$	$159\pm05^{@ns}$	$726\pm53^{@**}$
HFDF	$89\pm03^{\$ns}$	$132\pm07^{\$ns}$	$44\pm04^{\$ns}$	$101\pm07^{\$**}$	$93\pm10^{\$ns}$	$572\pm72^{\$*}$

NPDM-Normal pellet diet male, NPDF- Normal pellet diet Female, HFDM- High fat diet male, HFDF- High fat diet Female
[Values are expressed as mean ± SEM of 3 rats] *p<0.05; **p<0.01; ^{ns}-Non significant; Data was subjected to analysis through one way ANOVA with Sidak's multiple comparison test; @-comparison of HFDM with NPDM, $- comparison of HFDF with NPDF

Table 5: Creatinine (Kidney Marker) Levels

Group	Creatinine (mg/dL)	
	Day 0	Day 21
NPDM	0.44±0.03	0.40±0.01
NPDF	0.56±0.06	0.37±0.01
HFDM	$0.61\pm0.13^{@ns}$	$0.44\pm0.03^{@ns}$
HFDF	$0.53\pm0.02^{\$ns}$	$0.41\pm0.03^{\$ns}$

NPDM-Normal pellet diet male, NPDF- Normal pellet diet Female, HFDM- High fat diet male, HFDF- High fat diet Female [Values are expressed as mean ± SEM of 3 rats];^{ns}-Non significant; Data was subjected to analysis through one way ANOVA with Sidak's multiple comparison test; @-comparison of HFDM with NPDM, $- comparison of HFDF with NPDF

Table 6: Body Weight

Group	Day 0	Day 14	Day 21
NPDM	266±08	281±08	290±09
NPDF	220±06	235±06	243±09
HFDM	$258\pm11^{@ns}$	$315\pm11^{@*}$	$281\pm10^{@ns}$
HFDF	$231\pm02^{\$ns}$	$271\pm08^{\$*}$	$240\pm12^{\$ns}$

NPDM- Normal pellet diet male, NPDF- Normal pellet diet Female, HFDM- High fat diet male, HFDF- High fat diet Female [Values are expressed as mean ± SEM of 3 rats] *p<0.05; ^{ns}-Non significant; Data was subjected to analysis through one way ANOVA with Sidak's multiple comparison test; @-comparison of HFDM with NPDM, $- comparison of HFDF with NPDF

Table 7: Insulin (MIU/L) Levels

Group	Day 21
NPDM	6.8±0.2
NPDF	6.0±0.9
HFDM	$2.6\pm0.3^{@***}$
HFDF	$2.4\pm0.2^{\$**}$

NPDM- Normal pellet diet male, NPDF- Normal pellet diet Female, HFDM- High fat diet male, HFDF- High fat diet Female [Values are expressed as mean ± SEM of 3 rats] **p<0.01; ***p<0.001;^{ns}-Non significant; Data was subjected to analysis through one way ANOVA with Sidak's multiple comparison test; @-comparison of HFDM with NPDM, $- comparison of HFDF with NPDF

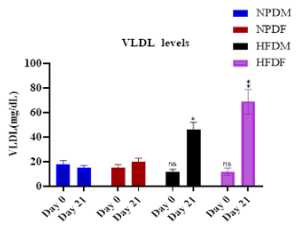

Figure 5: Serum VLDL Levels

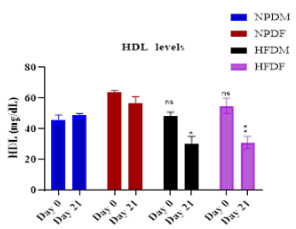

Figure 6: Serum HDL Levels

normal pellet diet animals on day 0 (p>0.05).

Two weeks of high fat diet feeding followed by streptozotocin administration resulted in significant elevation in ALT(p<0.01) & ALP(p<0.05) (p<0.01) levels, but there are no significant changes in AST levels were seen as compared to the NPD animals (p>0.05) [Table 4, Figure 7, Figure 8 and Figure 9].

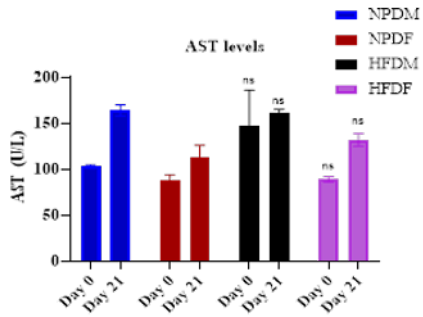

Figure 7: Serum AST Levels

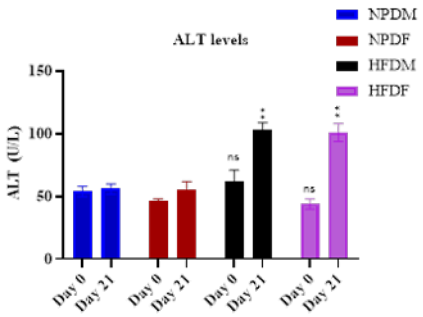

Figure 8: Serum ALT Levels

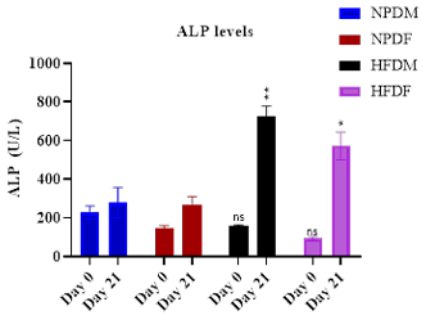

Figure 9: Serum ALP Levels

Kidney Markers

Creatinine as a kidney marker was measured in the serum. No significant changes creatinine levels were found in high fat diet animals as compared to the normal pellet diet animals on day 0 & day 21 ($p>0.05$) [Table 5 and Figure 10].

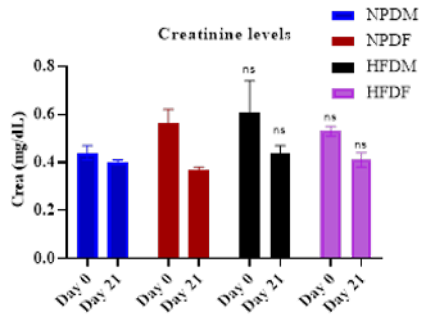

Figure 10: Serum Creatinine Levels

Body Weight

Post 2 weeks of high fat diet feeding Significant increase in body weight was observed in HFD group as compared to NPD group animals ($p<0.05$). However, streptozotocin administration reduced the body weight hence no significant changes in body weight were observed in HFD animals on 21^{st} day as compared to NPD group ($p>0.05$) [Table 6 and Figure 11].

Insulin

The insulin levels in the serum were measured by using ELISA kit. Streptozotocin intervention in high fat diet fed animals significantly lowered the insulin levels as compared to the NPD fed animals ($p<0.01$ & $p<0.001$) [Table 7 and Figure 12].

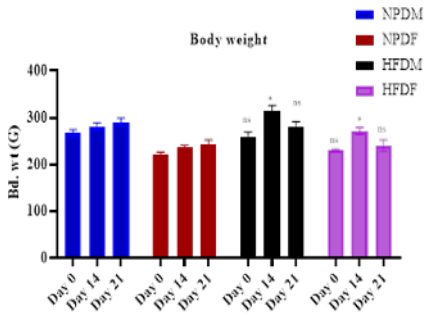

Figure 11: Body Weight

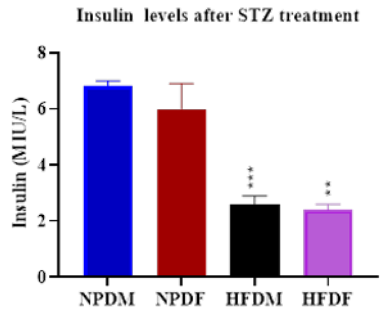

Figure 12: Serum Insulin Levels

DISCUSSION

The study was conducted with the objective of developing diabetic dyslipidaemia along with hepatocellular damage that would reflects natural history and metabolic characteristics of metabolic syndrome along with hepatic cirrhosis which responds to pharmacotherapy.

In this context, the male and female SD rats were fed with HFD and NPD for 2 weeks followed by single i.p injection of streptozotocin to HFD fed animals which produced the frank hyperglycaemia along with hypoinsulinemia similar to reported literature [6].

The high fat diet feeding produced the significant increase in bodyweight of HFD group rats as compared with NPD rats. Further the body weight was lowered post STZ treatment due to the catabolic loss induced by low dose STZ.

The development of rat model of metabolic syndrome along with hepatic damage was performed by feeding of HFD and administering low dose STZ. The high fat diet was procured from NIN, Hyderabad as per the composition described by

Sreenivasan et al. Streptozotocin induces pancreatic beta cell destruction by redox imbalance and subsequent oxidative stress [7]. This combination resulted in hyperglycaemia, hypoinsulinemia, hypercholesterolemia, hypertriglyceridemia and hyperlipidaemia that would closely reflects type-2 diabetes along with dyslipidaemia.

The diabetes also associated with hepato-cirrhosis and hepatocellular carcinoma (HCC) [8]. Liver is a key organ of regulation of glucose and lipid metabolism. In type-2 diabetes due to unopposed endogenous glucose production and insulin resistance which leads to hepatic damage caused by hepatic steatosis [9]. Hence the liver function tests such as AST, ALT & ALP are an essential part of clinical evaluation of a diabetic subject [10]. Correspondingly, the STZ administered HFD animals showed higher circulating levels of ALT&ALP which resembles hepatic cirrhosis in type-2 diabetic subjects.

Kidney also plays an important role in the regulation of blood glucose. The persistent high blood glucose levels result in diabetic nephropathy (DN) which is a progressive decline in renal function, and subsequent end Stage renal failure (ESRF) [11]. Creatinine is an important marker evaluating the function of kidney. Higher levels of blood creatine due to under excretion of creatinine has been observed in type 2 diabetes [12].

Based on the foregoing observations, the current model can be compared with the literature model by Sreenivasan et al. in the following aspects. Sreenivasan et al. initially developed the rat model of type-2 diabetes with HFD and low dose STZ. Sreenivasan et al. developed the model in male SD rats but the present study was conducted in male and female SD rats to avoid the variation due to sex on model validation.

Sreenivasan et.al described increase in body weight post STZ treatment in HFD fed animals but the current study establishes consistent decrease in body weight. Further the model was not validated on liver function. In contrary to this, present study the liver enzyme levels AST, ALT and ALP evaluated to establish the hepatic damage caused by STZ and HFD combination. Study also signifies the effect on kidney status by measuring the renal markers such as creatinine levels in serum.

CONCLUSION

Our study establishes that combination of HFD and single low dose STZ can effectively use a model for diabetic dyslipidaemia along with hepatic damage that reflects the natural history of metabolic syndrome in humans. It is an ideal model for type-2 diabetes along with hyperlipidaemia and hepatic cirrhosis and is useful in evaluating therapeutic compounds for metabolic syndrome.

Abbreviations

AST: Aspartate amino transferase, ALT: Alanine amino transferase, ALP= Alkaline phosphatase, CHO: Cholesterol, CREA: Creatinine, HFD= High fat diet, NPD= Normal pellet diet, HDL: High density lipoprotein, LDL: Low density lipoprotein, NIN: National Institute of Nutrition, OECD= Organization for economic cooperation and development, OGTT: Oral Glucose tolerance test, RH: Relative humidity, TRI: Triglycerides, VLDL: Very Low-density lipoprotein.

ACKNOWLEDGEMENT

The authors greatly acknowledge the Director General, Central Council for Research in Ayurvedic Sciences (CCRAS), New Delhi for providing intra mural research (IMR) funding and encouragement.

Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] R Nall. An overview of diabetes types and treatments. *Newsletter on Health. Medical News Today*, 1(1):1–5, 2018.

[2] M Asif. The prevention and control type-2 diabetes by changing lifestyle and dietary pattern. *Journal of education and health promotion*, 3, 2014.

[3] V Rajadhyaksha. Managing diabetes patients in India: Is the future more bitter or less sweet? *Perspectives in clinical research*, 9(1):1, 2018.

[4] A Kanno, S I Asahara, K Masuda, T Matsuda, M Kimura-Koyanagi, S Seino, and Y Kido. Compensatory hyperinsulinemia in high-fat diet-induced obese mice is associated with enhanced insulin translation in islets. *Biochemical and Biophysical Research Communications*, 458(3):681–686, 2015.

[5] K Srinivasan, B Viswanad, L Asrat, C L Kaul, and P Ramarao. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacological research*, 52(4):313–320, 2005.

[6] M J Reed, K Meszaros, L J Entes, M D Claypool, J G Pinkett, T M Gadbois, and

G M Reaven. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metabolism-Clinical and Experimental*, 49(11):51653–51662, 2000.

[7] S Lenzen. The mechanisms of alloxan-and streptozotocin-induced diabetes. *Diabetologia*, 51(2):216–226, 2008.

[8] M Trombetta, G Spiazzi, G Zoppini, and M Muggeo. type 2 diabetes and chronic liver disease in the Verona diabetes study. *Alimentary pharmacology and therapeutics*, 22:24–27, 2005.

[9] K F Petersen, S Dufour, D Befroy, M Lehrke, R E Hendler, and G I Shulman. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes*, 54(3):603–608, 2005.

[10] A Mandal, B Bhattarai, P Kafle, M Khalid, S K Jonnadula, J Lamicchane, and V Gayam. Elevated liver enzymes in patients with type 2 diabetes mellitus and non-alcoholic fatty liver disease. *Cureus*, 10(11), 2018.

[11] S Hussain, M C Jamali, A Habib, M S Hussain, M Akhtar, and A K Najmi. Diabetic kidney disease: An overview of prevalence, risk factors, and biomarkers. *Clinical Epidemiology and Global Health*, 9:2–6, 2021.

[12] N Harita, T Hayashi, K K Sato, Y Nakamura, T Yoneda, G Endo, and H Kambe. Lower serum creatinine is a new risk factor of type 2 diabetes: the Kansai healthcare study. *Diabetes care*, 32(3):424–426, 2009.