Ind. J. Pharm. Edu. Res., 2027; 61:10.5530/ijper.20270064.
https://www.ijper.org

Original Article

Antihyperlipidemic and Antidiabetic Effects of Valencene in Streptozotocin-Nicotinamide- Induced Type 2 Diabetic Rats

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ABSTRACT

Background: Dyslipidaemia, abnormal lipid metabolism and hepatic steatosis are common in patients with Diabetes Mellitus (DM) and contribute to the development of cardiovascular and metabolic complications. Valencene is a natural sesquiterpene with several pharmacological activities, however its effects on diabetic dyslipidaemia are not well elucidated. **Objectives:** To assess the antidiabetic and antihyperlipidemic effects of valencene in Streptozotocin-Nicotinamide (STZ-NA) induced diabetic rats in terms of lipid metabolism, lipid regulating enzymes and hepatic lipid accumulation. **Materials and Methods:** Nicotinamide (110 mg/kg BW: Body weight) followed by streptozotocin (45 mg/kg BW.) induced diabetes in rats. Diabetic rats were treated with glibenclamide (600 µg/kg BW) and valencene (200 mg/kg BW, p.o.) for 30 days. Plasma glucose, insulin, free fatty acids, Lipid Profile, Lipoprotein Lipase (LPL), Lecithin-Cholesterol Acyltransferase (LCAT), hepatic 3-Hydroxy-3-Methylglutaryl Coenzyme A (HMG-CoA) reductase activity, and hepatic lipid deposition were measured. **Results:** Valencene significantly decreased plasma glucose, free fatty acids, total cholesterol, triglycerides, phospholipids, LDL-C and VLDL-C and significantly increased plasma insulin and HDL-C levels. It also inhibited hepatic HMG-CoA reductase activity, increased the activities of LPL and LCAT and significantly reduced the accumulation of hepatic lipids and steatosis in diabetic rats. **Conclusion:** Valencene exhibited pronounced antidiabetic and antihyperlipidemic effects by enhancement of glucose homeostasis, regulation of lipid metabolism, inhibition of cholesterol synthesis, promotion of reverse cholesterol transport and amelioration of hepatic steatosis. These findings suggest valencene could be a potential therapeutic candidate for treating diabetic dyslipidaemia and related metabolic disorders.

Keywords: Diabetic rats, Hyperlipidemia, Nicotinamide, Streptozotocin, Valencene.

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Received: 22-07-2026;
Revised: 14-08-2026;
Accepted: 24-08-2026.

INTRODUCTION

A chronic metabolic disease, Diabetes Mellitus (DM), has grown to be a significant worldwide public health issue. According to the International Diabetes Federation's 2019 estimate, 463 million individuals globally have the disease, and by the year 2045, that figure may increase to about 700 million.[1] The hallmark of DM is hyperglycemia, which results from abnormalities in the metabolism of carbs, fats, and proteins, primarily from insufficient insulin production or decreased insulin sensitivity in pancreatic β-cells. This metabolic abnormality results in dysregulation of glucose and protein balance.[2]

Changes in lipid metabolism brought on by deficiencies in insulin biosynthesis and secretion, which have a key impact on important metabolic enzymes and pathways, are typically the hallmark of DM. Dyslipidemia is a frequently occurring complication of DM affecting more than 40% of patients.[3] Deranged metabolic regulation, particularly insulin deficiency, which is linked to elevated cholesterol and Triglyceride (TG) levels, is the cause of these lipid alterations.[4] DM has a significant impact on the liver, an insulin-dependent organ that is crucial to maintaining the balance between fats and carbohydrates. It takes up Free Fatty Acids (FFAs), oxidises them, and converts them to different lipid components, such as cholesterol, phospholipids, and triglycerides. Hepatic glucose metabolism is dramatically altered in DM with decreased glycolysis and glycogen synthesis and enhanced gluconeogenesis.[5]

Furthermore, DM significantly alters the composition and function. Patients with diabetes are more vulnerable to atherosclerosis as a result of all these alterations. One crucial underlying process is the increased mobilisation of triglycerides

DOI: 10.5530/ijper.20270064

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from adipose tissue, which elevates circulating Free Fatty Acid (FFA) levels, further aggravating lipid imbalance.[6]

Therefore, alternative therapeutic approaches to reduce cholesterol levels in patients with diabetes are important. Traditional medicinal plants contain alkaloids, terpenoids, flavonoids and phenolic derivatives which have shown promising hypoglycaemic and lipid lowering properties. Valencene is a naturally occurring sesquiterpene present in citrus fruits and has been reported to possess antihyperglycemic, antioxidant and anti-inflammatory activities among these phytochemicals. Previously, valencene was shown to stabilize glycoprotein components and exert antihyperglycemic and antioxidant effects in Streptozotocin-Nicotinamide (STZ-NA)-induced diabetic rats.[8,9] Antihyperglycemic and antioxidant benefits of valencene have been described in Streptozotocin-Nicotinamide (STZ-NA)-induced diabetic rats, however the present investigation is aimed at investigating the possible effects of valencene on diabetes-associated lipid dysregulation. Therefore, the anti-hyperlipidemic potential of valencene was evaluated by analysing plasma and hepatic lipid profiles viz. total cholesterol, triglycerides, phospholipids and free fatty acids, plasma lipoprotein fractions and the important lipid-regulating enzymes viz. Lipoprotein Lipase (LPL) and Lecithin-Cholesterol Acyltransferase (LCAT) and hepatic HMG-CoA reductase activity. In addition, hepatic lipid buildup and steatosis were histologically examined by Haematoxylin and Eosin (H&E) and Oil Red O staining. The present work combines these biochemical, enzymatic and histological endpoints to give a targeted assessment of the possible role of valencene in modifying diabetic dyslipidaemia and its related hepatic lipid accumulation in STZ-NA-induced diabetic rats.

MATERIALS AND METHODS

Drugs and chemicals

Nicotinamide (NA), valencene ((3R,4aS,5R)-4a,5-dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene, 98% purity), and Streptozotocin (STZ) were acquired from Sigma-Aldrich (USA: St. Louis, MO). HiMedia Laboratories and remaining chemicals and reagents were supplied by Mumbai's SD Fine Chemicals.

Animals and Diet

Healthy 180-210 g male albino rats (*Rattus norvegicus*) were donated by the Rajah Muthiah Medical College and Hospital, Central Animal House, Department of Experimental Medicine, Annamalai University, Annamalainagar, India. The animals had been held in typical laboratory conditions: 40 to 60% relative humidity, 25±2°C, with a 12-hr light/dark cycle. Rats received a typical commercial pellet food (Mumbai, India's Hindustan Lever Ltd.) and unrestricted access to water during the experiment. All experiments involving animals that adhere to the institution's ethical standards have been approved by the Institutional Animal Ethics Committee (IAEC) of Rajah Muthiah Medical College and Hospital at Annamalai University (CPCSEA Registration No. 160/1999; Proposal No. 1176/2017).

Induction of Diabetes Mellitus

DM was developed by experimentation in 180-210 g Body Weight (BW) *Rattus norvegicus* rats, starved overnight, by the streptozotocin-nicotinamide (STZ-NA) model. NA (110 mg/kg BW) in normal saline and freshly prepared STZ (45 mg/kg BW) in 0.1 M citrate buffer (pH 4.5) were administered intraperitoneally after 15 min. The animals received 20% glucose solution for a day, starting 6 hr after treatment, to avoid the consequences of acute insulin release following STZ administration. The study included animals with fasting blood glucose levels greater than 250 mg/dL, which were deemed to have Diabetes Mellitus (DM). Fasting blood glucose levels were measured 72 hr following the start of diabetes.

- **Group I:** Normal control rats.
- **Group II:** Normal + valencene (200 mg/kg/ BW).
- **Group III:** STZ +NA induced diabetic control rats.
- **Group IV:** Diabetic + valencene (200 mg/kg/ BW).
- **Group V:** Diabetic + glibenclamide (600 µg/kg/ BW).

Experimental Design and Diabetes Induction

Five groups of six rats each were created from the thirty rats. Twelve normal controls and eighteen surviving rats with diabetes caused by STZ-NA. Glibenclamide dissolved in distilled water was given intragastrically once daily for 30 days. Valencene was suspended in a 1 mL corn oil vehicle. Doses and duration followed prior work on valencene at 200 mg/kg for a month. Towards the end of the trial, rats that had fasted overnight were given intramuscular ketamine (24 mg/kg BW) to induce anesthesia before being beheaded. Jugular vein blood was drawn into tubes containing a 3:1 potassium oxalate:sodium fluoride anticoagulant for plasma glucose, insulin, and lipid tests. A portion of the liver was stored at -80°C for biochemical analysis. The remaining portion was quickly frozen in liquid nitrogen, while the other was preserved in 10% formalin for histological analysis.

BIOCHEMICAL ASSAYS

Insulin and plasma glucose measurements

Insulin and glucose levels were measured using ELISA kits from Boehringer-Mannheim, Mannheim, Germany, and commercial kits from Sigma Diagnostics Pvt. Ltd., Baroda, respectively. The Trinder (1969) approach, comparable to that used for systolic blood tests (Trinder *et al.* 1969), was used to estimate plasma

glucose. The manufacturer's instructions were followed for both analyses.[10]

Lipid Extraction

Folch *et al.* (1957) stated that chloroform-methanol (2:1, v/v) was used to extract tissues and plasma lipids. The samples were dried between the folds of filter paper after being cleaned with cold physiological saline. After a 24-hr extraction period, the tissues were homogenised in a chilled 2:1 chloroform: methanol mixture, and the extraction was performed 4 times. After mixing and cleaning the filtrates with a 0.7% KCl solution, the upper aqueous layer was discarded. TC, phospholipids, TGs, and FFAs were measured in aliquots after the chloroform phase was combined and added to a predetermined volume.[11]

Cholesterol Estimation

The Zlatkis, Zak, and Boyle (1953) method was used to measure total cholesterol (Zlatkis *et al.*, 1953).[12] After 15 min of incubation, Ferric chloride-acetic acid reagent (9.9 mL) and 0.1 mL of plasma or lipid extract were combined and centrifuged. Next, 3 mL of concentrated sulfuric acid (H_2SO_4) and 5 mL of the supernatant were added to a new tube. After 20 min of colour development, absorbance was measured at 560 nm relative to a reagent blank. The calibration curve was created by running a 200 mg/dL cholesterol standard solution in parallel.

Triglyceride Estimation

The Fossati and Prencipe (1982) method was used to measure triglyceride levels. Evaporation was used to dry 0.5 mL of lipid or plasma extract. After reconstituting the residue with 0.1 mL of methanol, 0.4 g of alumina and 4 mL of isopropanol were added. For 15 min, the mixture was vortexed before centrifugation. 2 mL of the supernatant was combined with the acetylacetone reagent (0.75 mL of acetylacetone in 60 mL of distilled water and 40 mL of isopropanol) and saponification reagent (5 g KOH dissolved in 60 mL of distilled water and 40 mL of isopropanol). Once the absorbance had cooled to room temperature, it was measured at 405 nm.[13]

Determination of FFAs

The Falholt, Falholt, and Lund (1973) technique was used to assay FFAs.[14] 50 µL of the sample-a mixture of heptane and methanol (49:49:2, v/v/v)-was added to a polyethene-stoppered test tube along with 1 mL of phosphate buffer (pH 6.4) and 6 mL of chloroform. The solution was agitated for 10 min and centrifuged at 2860 x g for 10 min. Following phase separation, 3 mL of the top layer was transferred to another tube containing 0.5 mL of a solution containing 10 mL of water and 4 g of 1,5-diphenylcarbazide. After that, the aqueous phase was precisely aspirated. The mixture was centrifuged for 5 min at 2,860 × g after 5mL of the organic phase was agitated with 2 mL of a copper reagent (0.05 M copper nitrate and 0.1 M triethanolamine, pH 8.1). After 15 min of color development, absorbance was measured at 550 nm. FFA levels were quantified in mg/dL in plasma and mg/100 g in tissue samples.

Phospholipid Determination

The Zilversmit and Davis (1950) method of converting organic phosphorus to inorganic phosphorus was used to quantify the Phospholipid (PL) level.[15] 0.1 mL of lipid extract or plasma was combined with 1mL of concentrated Sulfuric Acid (H_2SO_4) and Nitric Acid (HNO_3), and the mixture was digested until clarity was achieved. The inorganic phosphorus in the digest was subsequently quantified using the colorimetric method established by Fiske and Subbarow.[16]

Determination of the levels of the plasma lipid profile

Commercial testing kits from Beacon Diagnostics (Kabilpore, Navsari, India) were used to measure TC, HDL-C, TGs, LDL-C, and plasma. HDL-C was also measured using a reagent kit from Mumbai, India's Qualigens Diagnostics. The VLDL-C and LDL-C concentrations were determined using standard formulas:

$$\text{LDL} - \text{C} = \text{Total Cholesterol} - (\text{HDL} - \text{C} + \text{VLDL} - \text{C})$$

$$\text{VLDL} - \text{C is TG}/5$$

Lipid Marker Enzyme Estimation

Philipp and Shapiro's (1979) method was used to measure hepatic and renal 3-Hydroxy-3-Methylglutaryl Coenzyme A (HMG-CoA) reductase activity.[17] Equal volumes of 10% tissue homogenate were combined with diluted perchloric acid, left to stand for 5 min, and then centrifuged for 10 min at 2000 × g. 1.5 mL of ferric chloride solution was added after thoroughly combining 0.5 mL of alkaline hydroxylamine reagent with an aliquot of 1 mL of the supernatant. After 10 min, absorbance at 540 nm was measured using a saline-arsenate blank. Mevalonate to HMG-CoA was measured; a low ratio suggested higher enzyme function.

Plasma Lipoprotein Lipase (LPL) Activity

The technique developed by Korn (1955) was used to measure LPL activity.[18] 0.4 mL of substrate, 0.2 mL plasma, 0.1 mL of 1 M $CaCl_2$, 0.3 mL of 0.025 M NH_4Cl, 0.25 mL of 0.25 M ammonia buffer (pH 8.5), and an additional 0.1 mL $CaCl_2$ made up the reaction mixture. The reaction was halted after an hour of incubation at 37°C by adding 1 mL of ice-cold 0.1 M H_2SO_4. Before the addition of plasma, H_2SO_4 was added to control samples. The amount of glycerol released in the reaction mixture was then used to estimate LPL activity.

Plasma Lecithin-Cholesterol Acyltransferase (LCAT) Activity

The Hitz, Steinmetz, and Siest (1983) method was used to measure plasma Lecithin-Cholesterol Acyltransferase (LCAT) activity.[19] An equivalent volume of plasma was incubated with 0.6 mL of substrate. From this mixture, 0.2 mL was combined with 1mL of isopropanol and incubated for 90 min at 27°C. Using the Zlatkis *et al.* (1953) method, free cholesterol, representing the initial (zero-time) value, was measured in the supernatant after centrifugation. Isopropanol was added to a second aliquot of 0.2 mL of the reaction mixture after incubation at 27°C for 90 min.[12] The combination was incubated for an additional 90 min at the same temperature. After a total incubation period of 180 min and centrifugation, free cholesterol was again estimated by the same procedure. Substrate blanks without active plasma were included to confirm enzyme inactivation. LCAT activity was indicated by the decrease in free cholesterol during the incubation period.[20]

Liver Histopathology: Hematoxylin and Eosin (H&E) and Oil Red O Staining

After 48 hr of storage in 10% neutral buffered formalin, dehydration of liver tissues occurred in a series of graded ethanols before being embedded in paraffin wax. For traditional histological analysis, 5 µm sections were cut with a rotary microtome and stained with H&E. A light microscope was used to view the stained sections after they had been mounted. A sliding microtome was used to section liver samples at 30 µm to visualise lipids. Sections were submerged in 60% isopropanol after fixation in formalin, then rinsed with water. 30 mL of stock solution (0.5 g Oil Red O in 100 mL isopropanol) was diluted with 20 mL of distilled water to create a fresh Oil Red O working solution. Sections were stained with this solution for 20 min, counterstained with Mayer's hematoxylin, differentiated in 60% isopropanol, and mounted in aqueous medium. Finally, all sections were examined microscopically, and representative images were documented.

Statistical Analysis

All outcomes are displayed using the mean±Standard Deviation (S.D.). Duncan's Multiple Range Test (DMRT) and one-way Analysis of Variance (ANOVA) in SPSS version 25 were used to assess statistical significance between the experimental groups. If p-value<0.05, then the differences between the two experimental groups will be deemed statistically significant.[21]

RESULTS

Effect of Valencene on Insulin and Plasma Glucose

Diabetic control rats showed significantly higher plasma glucose levels (p<0.001) and significantly lower insulin levels (p<0.001) compared to normal control rats. Diabetic rats administered daily doses of glibenclamide or valencene for 30 days had significantly higher insulin levels (p<0.001) and significantly lower plasma glucose levels (p<0.001) compared to diabetic controls (Table 1).

Effect of Valencene on Plasma Lipids

STZ-NA diabetic control rats showed significantly higher TGs, TC, PLs, and FFAs in plasma and tissues in comparison to standard controls (p<0.001; Figures 1A-1D and Figures 2A-2D). Valencene treatment, along with glibenclamide, markedly reduced (p<0.001) these lipid levels in diabetic rats, restoring them toward normal values.

Effect of Valencene on Plasma Lipoproteins

Diabetic control rats (STZ-NA) had significantly lower HDL-C (p<0.001) and significantly higher LDL-C and VLDL-C (p<0.001) than normal control rats. Treatment with valencene and glibenclamide markedly normalized (p<0.001) the disrupted lipoprotein profile in diabetic rats, significantly enhancing it towards normal values (Figures 3A-3D).

Effect of Valencene on Plasma LPL and LCAT Activities

Compared with normal controls, STZ-NA diabetic control rats had significantly lower plasma LPL and LCAT activities (p<0.001). Valencene treatment, similar to glibenclamide, significantly restored both LPL and LCAT activities in diabetic rats (p<0.001) (Figures 4A and 4B).

Effect of Valencene on Hepatic HMG-CoA Reductase Activity

STZ-NA diabetic control rats showed substantially elevated hepatic HMG-CoA reductase activity compared to normal controls (p<0.001). Valencene and glibenclamide treatments significantly reduced (p<0.001) this elevated enzyme activity in diabetic rats (Figure 5).

Table 1: Effect of valencene on plasma glucose and insulin in experimental rats.

Groups	Glucose (mg/dL)	Insulin (µU/mL)
Normal control	86.70±6.63[a]	14.64±1.12[a]
Normal + valencene (200 mg/kg)	87.26±6.64[a]	14.29±1.08[a]
Diabetic control	256.76±19.55 [b]	5.72±0.43[b]
Diabetic + valencene (200 mg/kg)	108.47±8.30[c]	12.36±0.94[c]
Diabetic+glibenclamide (600 µg/kg)	98.86±7.56[a]	13.22±1.01[a]

Each value is mean±SD for six rats in each group. In each group, means with different superscript letter (a-c) differ significantly at p<0.05 (DMRT).

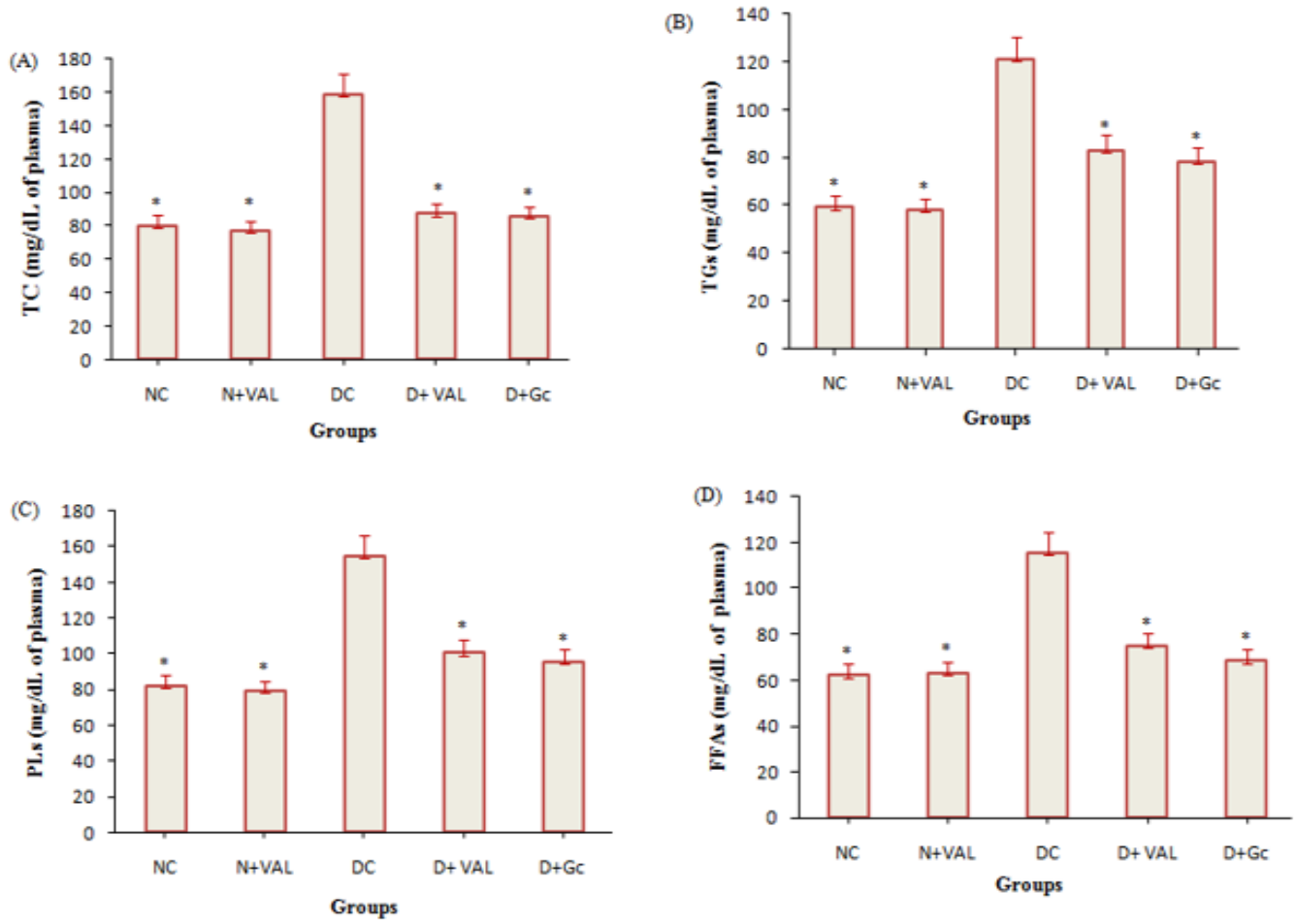


Figure 1: Effect of valencene on plasma levels of total cholesterol (A), triglycerides (B), phospholipids (C), and free fatty acids (D) in experimental rats. Each value is mean±SD for six rats in each group.*Significant difference compared with diabetic control group, $p<0.001$. NC: normal control; N+VAL: normal + valencene (200 mg/kg); DC: diabetic control; D +VAL: diabetic +valencene (200 mg/kg); D+Gc: diabetic+glibenclamide (600 µg/kg).

Hematoxylin and eosin and Oil Red "O" staining of the liver histopathology

Figure 6 Histopathological changes in the liver of experimental rats stained with Hematoxylin and Eosin (H&E) (A-E) and Oil Red "O" staining (F-J). (A) H&E-stained section of liver of normal control rats showed normal architecture of hepatocyte with intact cellular structure. (B) STZ-NA-treated diabetic control rats showed hepatic changes with increased hepatocellular degeneration and lipid accumulation. The liver sections of diabetic rats showed significant hepatocellular steatosis and fat accumulation (C). Diabetic rats treated with valencene (200 mg/kg body weight) exhibited a significant reduction in steatosis and improved hepatic architecture (D). Glibenclamide (600 µg/kg body weight)-treated diabetic rats showed almost normal hepatic morphology with less pathological changes (E). Oil Red "O" staining showed increased accumulation of lipid droplets in liver sections of diabetic control rats (H) induced by STZ-NA. Treatment with valencene (200 mg/kg body weight) significantly reduced hepatic lipid accumulation and showed near normal distribution of lipids (I). There was minimal deposition of lipids in diabetic rats treated with glibenclamide and the pattern was similar to normal liver sections (J). There was minimal lipid accumulation in normal control rats (F).

DISCUSSION

An established experimental model for evaluating potential hypoglycemic medications is the STZ-NA-induced hyperglycemia model. Our earlier study showed that administering valencene (200 mg/kg body weight) to diabetic rats significantly improved glycemic control and elevated plasma insulin levels. The activation of the remaining pancreatic β-cells, which are essential for insulin secretion, may be the cause of this effect. Furthermore, circulating and tissue lipid levels, as well as insulin sensitivity, are inversely correlated.[22] In the present research, we examined valencene's potential antihyperlipidemic effects in rats with artificially induced DM.

Metabolic dyslipidemia is the most common adverse effect linked to Type 2 Diabetes (T2D) and insulin resistance. In individuals with DM, a metabolic disorder known as dyslipidemia increases

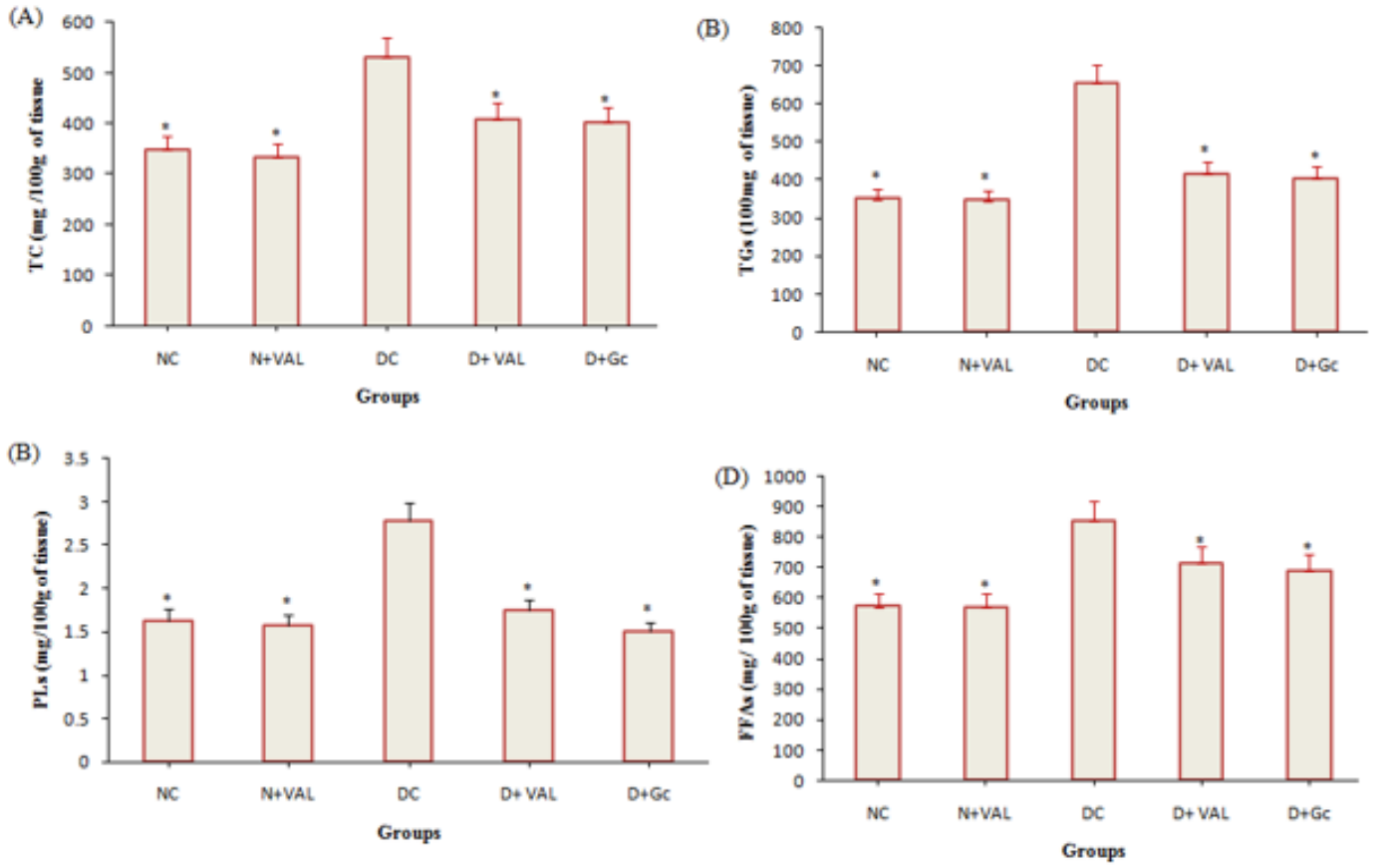


Figure 2: Effect of valencene on hepatic levels of total cholesterol (A), triglycerides (B), phospholipids (C), and free fatty acids (D) in experimental rats. Each value is mean±SD for six rats each group. *Significant difference compared with diabetic control group $p<0.001$. NC: normal control; N+VAL: normal + valencene (200 mg/kg); DC: diabetic control; D +VAL: diabetic +valencene (200 mg/kg); D+Gc: diabetic+glibenclamide (600 µg/kg).

the risk of atherosclerosis and cardiovascular disease.[23] Research has demonstrated that insulin insufficiency leads to lipid buildup in diabetic individuals, encompassing TC and TGs. When there isn't enough insulin, the body breaks down too many lipids in fat stores, which boosts Free Fatty Acid (FFA) levels.[23] Uncontrolled diabetes mellitus is associated with higher levels of TC, TGs, VLDL cholesterol, and lower levels of HDL cholesterol. All of these things can lead to coronary artery disease.

The most prevalent complication of insulin resistance and T2D is metabolic dyslipidemia. Dyslipidemia is a metabolic abnormality that heightens the risk of atherosclerosis and cardiovascular disease in individuals with DM.[24] Insulin deficiency causes lipid accumulation in diabetic patients, including total cholesterol and TGs. Insulin deficiency leads to excessive lipid breakdown in adipose tissue, raising FFA levels.[25] Uncontrolled DM correlates with high levels of LDL, TGs, TC, and VLDL cholesterol, alongside a reduction in HDL cholesterol, all of which contribute to coronary artery disease.

This study demonstrated that valencene administration effectively reduced TC and TG levels in diabetic rats, indicating an optimal metabolic condition for atherosclerosis prevention. Other studies indicate that diosmin, a terpene, reduces TC and TG levels by inhibiting cholesterol absorption and altering lipid-metabolism enzymes.[26]

Plasma and hepatic lipid levels were considerably higher in the STZ-NA-induced diabetic rats used in this investigation. FFAs are an essential source of energy for heart, liver, kidney tissues, and skeletal muscle. On the other hand, elevated FFA levels promoted NADPH synthesis, NADPH-dependent microsomal lipid peroxidation, and consequently oxidative stress.[27]

Phospholipids (PLs) are the primary structural elements of surface lipoproteins and are essential for cell membrane integrity. These lipid bilayers separate the non-polar lipoprotein core from the surrounding polar plasma environment. PLs are particularly vulnerable to damage caused by free radicals, including reactive Oxygen species like superoxide ($O_2\bullet^-$) and Hydroxyl radicals (•OH), content of Polyunsaturated Fatty Acids (PUFAs). Additionally, PLs are crucial for the transport of triglycerides. Increased PL levels are generally associated with enhanced mobilization of FFAs.[28] The normalisation of FFA and PL levels observed in valencene treatment may be associated with increased insulin secretion and sensitivity, as well as with regulation of FFA release. Insulin increases glucose utilisation

while suppressing FFA mobilisation from adipose tissue by inhibiting hormone-sensitive lipase activity.

Dyslipidemia is generally characterised by decreased HDL-C levels and increased VLDL-C and LDL-C levels. These changes may be associated with impaired clearance of VLDL, caused by reduced lipoprotein lipase activity, decreased LDL uptake due to glycation of ligand proteins, and increased hepatic secretion of VLDL particles, prompted by increased free fatty acid and glucose levels. The increase in LDL-C could also be related to the glycation of lysine residues on apolipoprotein B, which reduces its binding affinity for LDL receptors. In this sense, valencene could help restore normal lipoprotein profiles by increasing lipoprotein lipase activity and limiting lipoprotein glycation.[29]

The majority of circulating Cholesteryl Esters (CEs) are formed by the plasma enzyme Lecithin-Cholesterol Acyltransferase (LCAT).[30] By catalyzing the esterification of free cholesterol within HDL particles and promoting the return of VLDL and LDL remnants to the liver, LCAT plays a critical part in reverse cholesterol transport. Unesterified cholesterol builds up in cell membranes, and remnant lipoproteins accumulate in plasma as a result of LCAT inactivation, both of which are independently associated with the development of atherosclerosis.[31] HDL is the primary substrate for LCAT, providing phosphatidylcholine (lecithin) as the donor molecule and apolipoprotein A-I (Apo A-I) as an important activator.[32] LCAT also promotes HDL maturation and function by esterifying free cholesterol. In chronic hyperglycemia, as in DM, non-enzymatic protein glycation (e.g., Apo A-I) occurs, which diminishes Apo A-I's ability to activate LCAT and thereby reduces enzyme activity.[33] Additionally, elevated blood glucose may directly glycate LCAT, leading to loss of enzymatic function. Compared with untreated diabetic controls, the current study showed that valencene treatment in diabetic rats increased plasma LCAT activity.

An essential enzyme in cholesterol biosynthesis is hepatic 3-Hydroxy-3-Methylglutaryl-Coa (HMG-CoA) reductase.[34] Its activity is often elevated in diabetic conditions, probably due to insulin deficiency, leading to increased endogenous cholesterol production. This increased activity is usually expressed as a

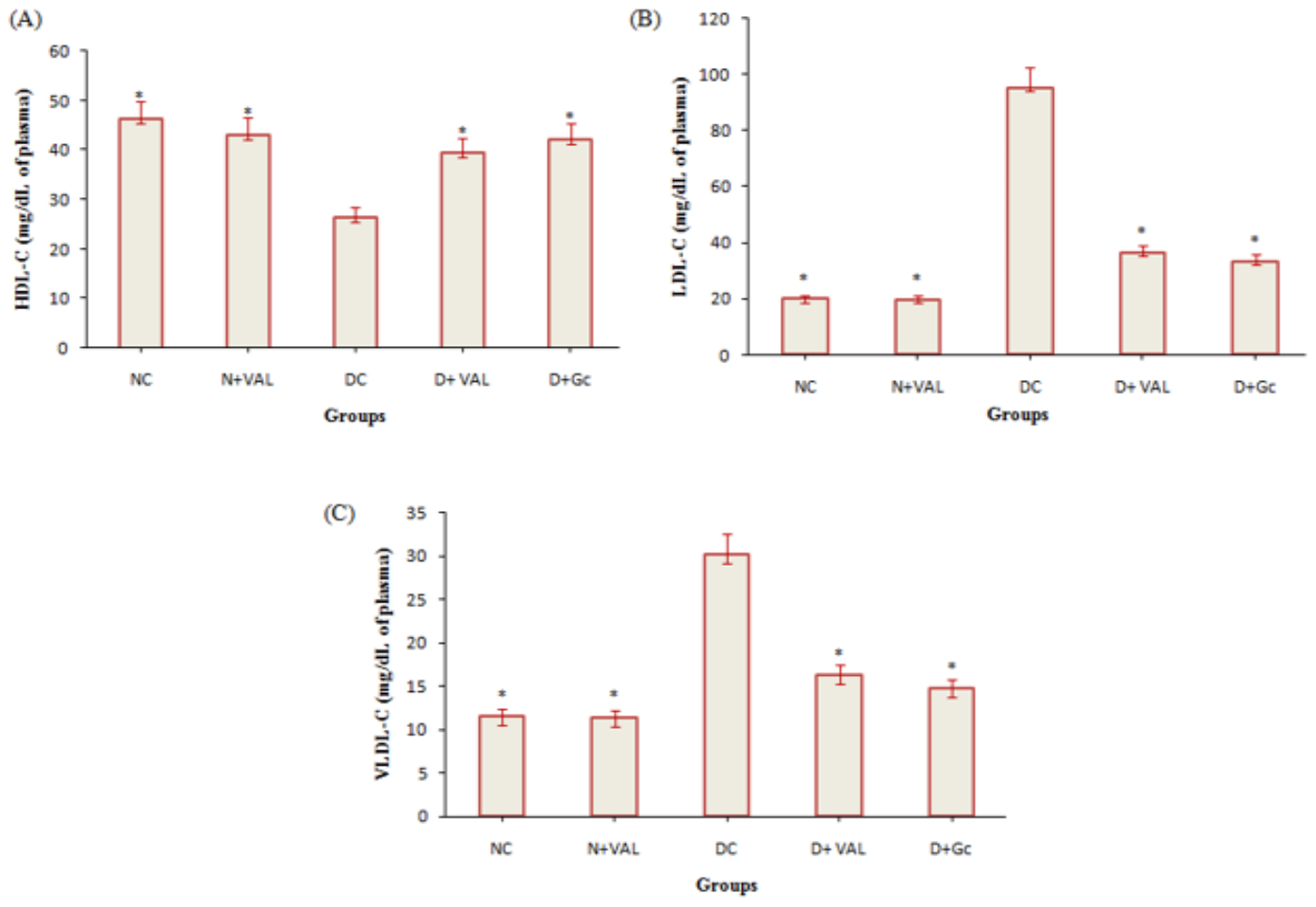


Figure 3: Effect of valencene on plasma lipoprotein levels in experimental rats.Each value is mean±SD for six rats in each group. *Significant difference compared with diabetic control group $p<0.001$. NC: normal control; N+VAL: normal + valencene (200 mg/kg); DC: diabetic control; D +VAL: diabetic +valencene (200 mg/kg); D+Gc: diabetic+glibenclamide (600 µg/kg).

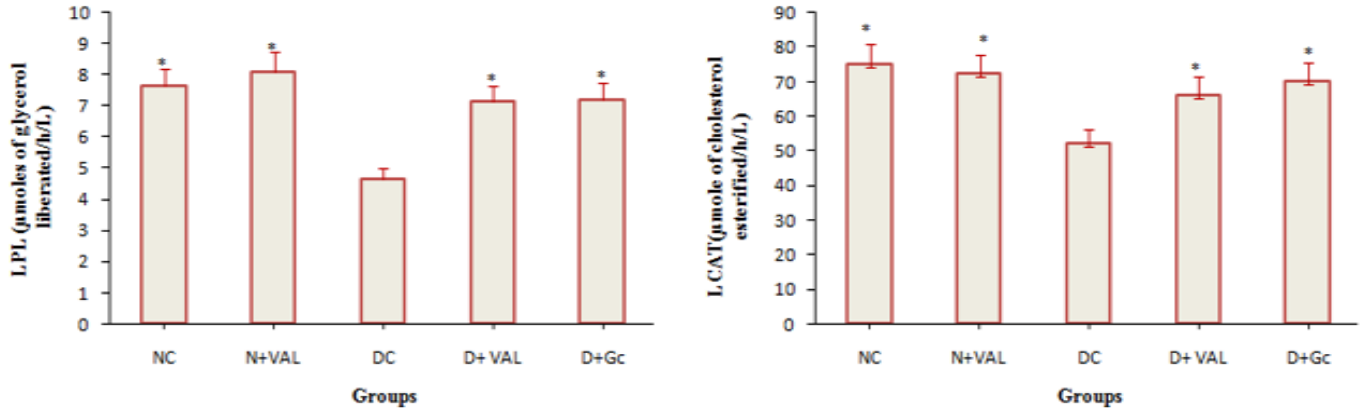


Figure 4: Effect of valencene on plasma Lipoprotein Lipase (LPL) (A) and Lecithin-Cholesterol Acyltransferase (LCAT) (B) activities in experimental rats. Each value is mean±SD for six rats in each group. *Significant difference compared with diabetic control group $p<0.001$. NC: normal control; N+VAL: normal + valencene (200 mg/kg); DC: diabetic control; D +VAL: diabetic +valencene (200 mg/kg); D+Gc: diabetic+glibenclamide (600 µg/kg).

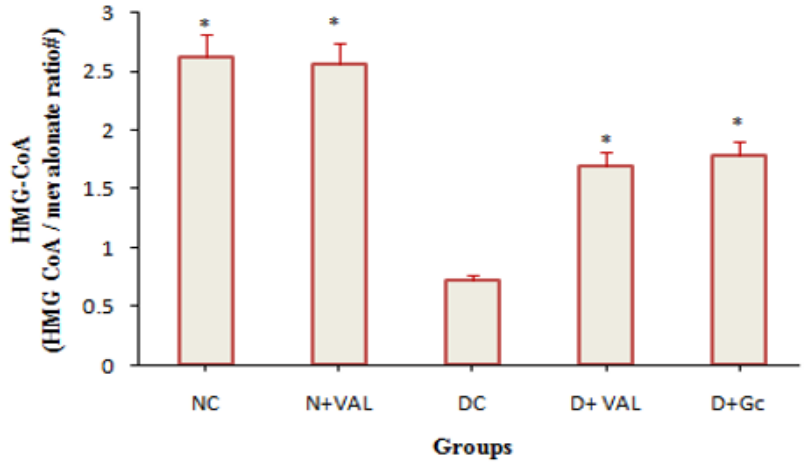


Figure 5: Effect of valencene on hepatic HMG-CoA reductase activity in experimental rats.Each value is mean±SD for six rats in each group. *Significant difference compared with diabetic control group $p<0.001$. #Lower ratio indicates higher enzyme activity, and higher ratio indicates lower enzyme activity. NC: normal control; N+VAL: normal + valencene (200 mg/kg); DC: diabetic control; D +VAL: diabetic +valencene (200 mg/kg); D+Gc: diabetic+glibenclamide (600 µg/kg).

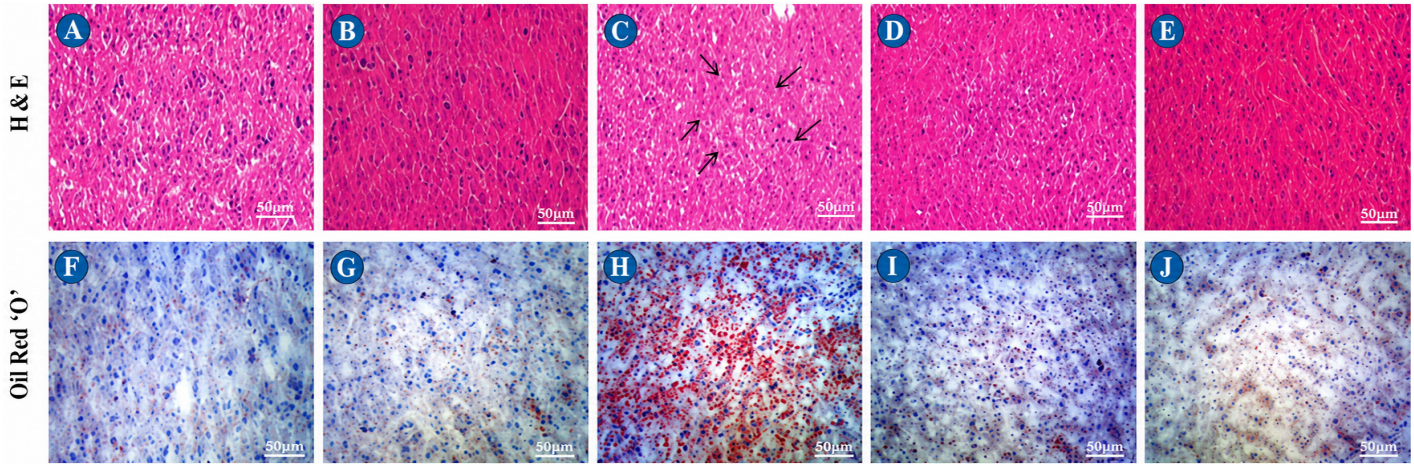


Figure 6: Histopathological changes in the liver of experimental rats stained with Hematoxylin and Eosin (H&E) (A-E) and Oil Red O (F-J). (A, F) Normal control; (B, G) normal + valencene; (C, H) diabetic control; (D, I) diabetic + valencene; and (E, J) diabetic + glibenclamide.

reduced HMG-CoA/mevalonate ratio. In this study, diabetic rats induced by STZ-NA exhibited significantly increased hepatic HMG-CoA reductase activity compared to normal control animals. However, valencene treatment significantly decreased the enzyme activity in diabetic rats, inhibition of hepatic cholesterol synthesis. The observed changes in LPL, LCAT and hepatic HMG-CoA reductase activity suggest an effect of valencene on lipid metabolism, but the present study did not evaluate upstream molecular signalling pathways such as PPARs, AMPK or SREBP-1c. Therefore, the specific molecular mechanisms of the antihyperlipidemic actions of valencene demonstrated in the present study should be elucidated. Further investigations, including molecular and signalling pathway analysis, are needed to clarify these pathways.

The accumulation of lipids in the liver impairs hepatocyte function and, as a result, produces liver glucose and hyperglycemia. Therefore, we studied the effect of valencene on hepatic fat accumulation in drug-administered rats. Lipid droplet accumulation was seen in the livers of STZ-NA-induced diabetic rats; however, this accumulation was significantly reduced in the livers of valencene-treated STZ-NA-induced diabetic rats, indicating that valencene treatment may reverse steatosis (A-J). However, diabetic rats given oral valencene and glibenclamide showed greater fat accumulation.

The sulfonylurea receptor, an inhibitory subunit of pancreatic β-cells' ATP-Sensitive Potassium (KATP) channels, is bound and activated by glibenclamide. This stimulates insulin secretion, elevates intracellular calcium levels in β-cells, activates voltage-gated calcium channels, and inhibits depolarisation of the cell membrane.[35] Sesquiterpene molecule resveratrol controls blood glucose levels and inhibts Body Weight (BW) its ATP-sensitive K^+ channels. A sesquiterpene molecule called valencene may increase intracellular calcium ions and stimulate insulin release in remaining β-cells while blocking ATP-sensitive K^+ channels. However, more research on the valencene process is required.

A limitation of the present study is that only single dose (200 mg/kg body weight) of valencene was tested. Hence, the dose-response relationship for the antidiabetic and antihyperlipidemic activities of valencene could not be elucidated. There is need for further study using multiple doses in order to establish the dose-dependent effect of valencene and also to evaluate the most effective dose.

CONCLUSION

Valencene administration markedly improved lipid metabolism in rats with diabetes caused by STZ-NA, as evidenced by normalization of lipid profile measurements, including TGs, TC, HDL-C, FFAs, VLDL-C, LDL-C, and PLs, and by increases in plasma and tissue levels of lipid-regulating enzymes. The results suggest that valencene has strong antihyperlipidemic properties and may be preventive against diabetes-associated dyslipidemia. Therefore, valencene may be considered a promising medicinal agent for the control and prevention of lipid abnormalities associated with problems from DM.

ABBREVIATIONS

DM: Diabetes mellitus; **STZ-NA:** Streptozotocin-nicotinamide; **T2D:** Type 2 diabetes; **TGs:** Triglycerides; **FFAs:** Free fatty acids; **KATP:** ATP-sensitive potassium channels; **CEs:** Cholesteryl esters; **PUFAs:** Polyunsaturated fatty acids; **BW:** Body weight; **LPL:** Lipoprotein lipase; **LCAT:** Lecithin cholesterol acyltransferase; **HMG-CoA:** 3-Hydroxy-3-methylglutaryl A; **TC:** Total cholesterol; **PL:** Phospholipids; **LDL-C:** Low-density lipoprotein cholesterol; **HDL-C:** High-density lipoprotein cholesterol; **VLDL-C:** Very-low-density lipoprotein cholesterol.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Paari Ellappan: Conceptualization, Investigation, Methodology, Data curation, Writing - Original draft. **Neelakandan Mani:** Methodology, Validation, Formal analysis. **Rajasekaran Duraisamy:** Supervision, Review and Editing. **Pari Leelavinothan:** Conceptualization, Supervision, Project administration, Writing - Review and Editing.

ETHICAL APPROVAL

All experimental procedures involving animals were performed in accordance with the institutional ethical guidelines and approved by the Institutional Animal Ethics Committee (IAEC), Rajah Muthiah Medical College and Hospital, Annamalai University. The study was approved by CPCSEA Registration No. 160/1999 and Proposal No. 1176/2017 and all animal experiments were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

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Cite this article: Ellappan P, Mani N, Duraisamy R, Leelavinothan P. Antihyperlipidemic and Antidiabetic Effects of Valencene in Streptozotocin-Nicotinamide-Induced Type 2 Diabetic Rats. Indian J of Pharmaceutical Education and Research. 2027;61:10.5530/ijper.20270064.