

METFORMIN AND CAFFEINE'S INFLUENCE ON LIPID PROFILE INDICES OF STREPTOZOTOCIN-INDUCED DYSLIPIDEMIA IN AN ANIMAL MODEL

Original Research Article

ABSTRACT

Dyslipidemia has been linked to insulin resistance and hyperglycemia. Hyperglycemia, which is the outcome of either insufficient insulin production or insensitivity to insulin, is the hallmark of diabetes mellitus (DM). In this study, thirty-six (36) male Wistar rats with diabetes induced by streptozotocin were used to determine the effect of metformin and caffeine on blood glucose levels and lipid profile indices. The rats weighed between 150 and 200 grams and were split into six groups of six animals each at random. Group A was given unlimited access to chow (Grower feed) and water ad libitum. Group B was administered 60 mg/kg of streptozocin (STZ) without any medication, while Group C was given 60 mg/kg of STZ together with 50 mg/kg of metformin. Groups D and E were administered 60 mg/kg of streptozocin and 25 mg/kg and 50 mg/kg of caffeine, for a duration of 21 days respectively. Group F was given 50 mg/kg of caffeine together with 50 mg/kg of metformin for a duration of 21 days. After performing a post-hoc LSD comparison and using ANOVA to analyze the results, the lipid profile and blood glucose level were deemed significant at $p < 0.05$. A paired t-test was used to analyze body weight. Rats with diabetes had reduced body weight, whereas those receiving metformin and caffeine treatments had noticeably increased body weight. The findings demonstrated that whereas metformin and caffeine therapy greatly reduced blood glucose levels, STZ caused hyperglycemia in group B. However, diabetic rats had considerably higher levels of triglycerides (TG) and total cholesterol (TC), which were reduced by metformin and caffeine administration. HDL activity was considerably increased after treatment with metformin and caffeine, despite the rats' reduced HDL levels in group B. Rats with diabetes had considerably elevated levels of LDL and VLDL, and therapy with metformin and caffeine markedly restored their activity. The study shows that caffeine plus metformin may be utilized to control the lipid profile and blood glucose levels in diabetic rats.

Keywords: Caffeine, Metformin, Streptozotocin, Diabetes Mellitus, lipid profile

Introduction

Hyperglycemia, which is the outcome of either insufficient insulin production or insensitivity to insulin reduction, is the hallmark of diabetes mellitus (DM), a complex illness. According to Tan et al., 2019, and Green et al., 2021, DM is highly prevalent, and the primary cause of high mortality and morbidity rates worldwide, which directly affects the liver, increasing the generation of glucose

in the liver as a result of insulin loss (Ifedi et al., 2021). The reports of Saeedi et al. (2019), demonstrated that poor glucose tolerance is expected to affect 7.5% of people (374 million) in 2019 and increase to 8.0% of people (454 million) by 2030 and 8.6% of people (548 million) by 2045. Moreover, diabetes mellitus has a strong racial component and is brought on by a number of hereditary or environmental variables. Genetic in nature, DM is located on chromosome 16 and affects around 57 genes with 136 single nucleotide polymorphisms (SNPs). Type 2-DM, which accounts for a higher percentage (87%) of disability in low- and middle-income countries, is a metabolic disease connected to dyslipidemia, hypertension, and hyperglycemia. These conditions are considered to be substantial modifiable risk factors for DM (Yusuf et al., 2014).

A naturally occurring substance called streptozotocin (STZ) is obtained from *Streptomyces achromogenes*. It is especially harmful to the beta cells of mammals' pancreas that produce insulin. It is a poison that damages DNA and produces free radicals, which permanently harm pancreatic islets (Tan & Ong, 2014). Streptozotocin is an alkylating agent and glucosamine-nitrosourea combination that damages DNA, particularly in islet cells. Carbonium ions (CH_3^+), which alkylate purine and pyrimidine bases to generate DNA adducts, are the byproduct of streptozotocin's spontaneous breakdown. It is well established that the STZ causes insulin-dependent diabetes by breaking down DNA and destroying beta cells. The beta-cell mitochondrial metabolism's signalling function is compromised by targeting mitochondrial DNA. Research has demonstrated that streptozotocin degrades pancreatic beta-cells, leading to diabetes in animal models (Akinlade et al., 2021; Furman, 2021). Moreover, Akinlade et al. (2021) observed that in type 2 diabetic rats, STZ significantly raised the levels of triglycerides, total cholesterol, and fasting blood glucose. Blood As the primary energy source for both aerobic and anaerobic respiration, glucose is also thought to provide the fuel for healthy bodily physiology. There are various kinds of glucose that the body uses and gets from the breakdown of fats and proteins in the liver through a process called

gluconeogenesis (Gurung & Jialal, 2021). The regulation of glucose occurs through intricate pathways that are influenced by a variety of hormones (Qi & Tester, 2019a). Hyperglycemia and hypoglycemia are clinical conditions where blood glucose levels are either above or below normal physiological values. They are caused by glucose pathology. However, the hyperglycemia phase, which can be acute or chronic and affects people with diabetes mellitus I and II, is characterised by a spike in blood sugar levels brought on by inadequate glucose management, which increases the risk of serious complications (Dhatariya et al., 2020). Moreover, a confluence of immunological, environmental, and genetic factors—most prevalent in paediatric patients—causes type I diabetes (Yau et al., 2021). On the other hand, type II appears later in life and is linked to comorbid diseases like obesity and hereditary factors. As shown in type II diabetes, beta cell death is the main mechanism responsible for elevated blood glucose levels (Christensen and Gannon, 2019); this death is linked to interactions between many molecular pathways and the environment. Additionally, it has been documented that obesity-related lipotoxicity, glucotoxicity, and glucolipotoxicity cause oxidative stress and metabolic impairment that damages β -cells and impairs beta-cell function normally, favouring insulin resistance as the main cause of type II diabetes. In addition, the β -cells are exposed to ER stress, inflammatory stress, metabolic/oxidative stress, and amyloid stress due to the aforementioned conditions, which may ultimately result in the loss of islet integrity (Christensen and Gannon, 2019; Galicia-Garcia et al., 2020). The process of restoring hyperglycemia is multifaceted, involving extensive neuro-hormonal regulation and counter-regulatory mechanisms. Insulin-dependent and -independent mechanisms play a role in the control of postprandial plasma glucose and fasting. Insulin regulates hormones by attaching to receptors in muscle, adipose tissue, liver, and kidney. Insulin then uses the glucose transporter systems in these organs to restrict the release of glucose (Charles et al., 2022, Tao & Cheng, 2023). Overall cholesterol, triglycerides, LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C) make up the majority of the lipid profile, which is categorised as a diagnostic tool for cardiovascular risk factors

(Dasgupta & Wahed, 2021). According to Dasgupta & Wahed (2021) lipids are the main components that give mammalian cells their functions and are referred to as the important building blocks of life. A high amount of lipids in the blood, such as cholesterol and triglycerides, known as hyperlipidemia, raises the risk of developing atherosclerotic cardiovascular disease (CVD) and is connected to the progression of cardiovascular disease. Cholesterol plays a crucial role in this process (Lee & Siddiqui, 2022). Dyslipidemia has been associated with diabetes mellitus (DM), which is a modifiable risk factor for cardiovascular complications in type 2 DM (Haile & Timerga, 2020). It is distinguished by elevated levels of low-density lipoprotein particles, decreased levels of HDL cholesterol, and hypertriglyceridemia. According to Haile and Timerga (2020), dyslipidemia has been linked to insulin resistance and hyperglycemia, which can lead to an abnormal postprandial lipoprotein metabolism, low clearance of triglyceride-rich lipoproteins, and an excess of triglyceride-rich lipoproteins produced by the liver. According to Khatana et al. (2020), elevated triglyceride activity changes the triglyceride content and particle sizes of low-density lipoproteins (LDLs), a phenomenon linked to heightened atherogenicity.

One of the most popular psychoactive chemicals used worldwide is still caffeine. Nonetheless, the global average for its consumption is between 70 and 76 mg/person per day; however, in several western regions, such as North America, Sweden, and Finland, this amount can reach up to 210 mg/day (Blaise et al., 2018, Arthur et al., 2018). The natural alkaloid methylxanthines that include caffeine, theophylline, and theobromine are commonly present in foods and drinks such coffee, tea, chocolate, energy drinks, and carbonated drinks like cola and other soft drinks (Blaise et al., 2018). As an antidiabetic, metformin is regarded as the first line of treatment for type 2 diabetes. It has been connected, seemingly independently of the medication's ability to lower blood sugar, to improved glucose levels as well as other conditions related to diabetes, including lipids, inflammatory markers, and a decrease in cardiovascular events (Sanchez-Rangel & Inzucchi, 2017).

A biguanide derivative called metformin regulates blood sugar levels and lessens related problems; however, it does so by reducing blood sugar levels through non-pancreatic processes rather than by raising insulin production (Petersen & Shulman, 2018). It is referred to as a “insulin sensitizer” since it amplifies the effects of insulin. According to Nasri and Rafieian-Kopaei (2014), metformin also inhibits the liver's natural synthesis of glucose. This is mostly because it slows down the pace of gluconeogenesis and has no effect on glycogenolysis. According to a report, in type 2 diabetic rats, coffee—the main source of caffeine—improves insulin sensitivity and glucose tolerance. Additionally, in diabetic mice, coffee was linked to decreased levels of low-density lipoprotein and triglycerides (Morakinyo et al., 2013). According to research by Akash et al. (2014), giving diabetic rats coffee caused their blood glucose levels to significantly drop. While Kempf et al. (2010) observed a significant shift in low-density lipoprotein and high-density lipoprotein levels and their ratios, which was attributable to the caffeine, they also revealed a minor change in the glucose tolerance test in diabetic rats. In male rats with alloxan-induced diabetes, Akinola et al. (2019) demonstrated the anti-lipidemic benefit of caffeine-containing beverages. According to Feyisa et al. (2019), coffee dramatically lowers blood glucose, triglycerides, total cholesterol, low-density lipoprotein, and HDL-C levels. By affecting the level of serum total bile acids, metformin may aid in the improvement of lipid and glucose metabolism (Meng et al., 2017). Metformin was found to reduce insulin levels and lipid changes in rats fed high fructose, according to Anurag and Anuradha (2002). The study aims to look into the acute and chronic phases of the effects of caffeine, which are less well documented in the literature than the attenuated effects on glucose level and lipid profile in diabetic rats, which have been the subject of several papers.

Materials and method

Chemicals and Reagents Procurements

Caffeine (CDH Chemicals, New Delhi, India), Chloroform (Guangdong, China), 200 mmol/liter phosphate buffer, pH 7.5, containing glucose oxidase (EC 1.1.3.4) >11 U/ml; peroxidase (EC 1.11.1.7) >0.02 U/ml; 4-aminophenazone 0.77 mmol/liter; phenol 11 mmol/liter.

Experimental Animals

Thirty-six-(36) male Wistar rats weighing 150-200g was obtained from the Animal House, College of medicine, University of Nigeria, Enugu Campus. Animals were kept in standard cages at a room temperature of $27\pm 2^{\circ}\text{C}$. The animals were maintained with normal laboratory chow (Grower feed) and water *ad libitum*. The animals were acclimatized for two weeks and before the induction of diabetes and caffeine administration. The animals were kept on 12hours light and dark cycles.

. Experimental design

The animals were divided into six-groups of six animals per group as follows:

Group A received feed and water *ad libitum* only for 21 days

Group B receive 60mg/kg of Streptozocin only without treatment for 21 days

Group C received 60mg/kg of Streptozocin and treated with 50mg/kg of metformin for 21 days

Group D received 60mg/kg of Streptozocin and treated with 25mg/kg of caffeine for 21 days

Group E received 60mg/kg of Streptozocin and treated with 50mg/kg of caffeine for 21 days

Group F received 50mg/kg of metformin and 50mg/kg of caffeine for 21 days

Induction of diabetes

Diabetes Mellitus (Hyperglycemia) was induced in the experimental rats by a single dose of intraperitoneal injection of 60mg/kg of Streptozocin. The animals were fasted 12-hours prior to the

induction of diabetes. Hyperglycemia was confirmed when fasting blood glucose concentration was greater than 200mg/dL for three consecutive days as described by Charles et al., (2021), Akbarzadeh et al. (2007).

Acute toxicity of Caffeine

The median lethal dose (LD₅₀) of Caffeine was carried out in the department of Physiology, College of Medicine, University of Nigeria, Enugu Campus. This was determined using the method of Lorke (1983). Thirteen-(13) rats were used in the determination of the LD₅₀ of Caffeine and will be grouped into two phases: Phase I consist of three groups of three rats each, and phase II consists of four groups of one rat each.

Determination of Blood Glucose Levels

Estimation of blood glucose level was done using the accu-check glucometer and strips. Blood was obtained from the tail vein of the rats. After the commencement of treatments, the blood glucose level was determine in all the groups at day 0, 7, and 14 by using the Glucose oxidase method approached using the enzymatic method described by Duxbury (2006) and Trinder (1969).

Glucose oxidase method

Glucose is oxidized by glucose-oxidase (GOD) in the presence of atmospheric oxygen to gluconolactone and hydrogen peroxide. The hydrogen peroxide is then oxidized by peroxidase (POD) in the presence of 4-aminophenazone and phenol to form the red dye 4-(*p*-benzochinone-monoimino)-phenazone, which is quantitated spectrophotometrically at 505 nm.

Collection of blood samples

At the end of the experiment, animals in the different groups were anesthetized using chloroform in an enclosed container after 24-hours of the last administered dose of caffeine. Blood was collected

from the animals using a heparinized capillary tube through ocular puncture as described by Parasuraman et al.,(2010) and Charles *et al.*, (2021). Blood obtained was put in a plain bottle, allowed to clot, and centrifuged for 10-minutes at 3000rpm, after which the serum was retrieved using a micropipette. The retrieved serum was used to assay for fasting blood glucose level, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglyceride, and very low-density lipoprotein.

Determination of Lipid profile

Determination of Total cholesterol

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol (Li et al., 2019). One of the reaction byproducts, H₂O₂ is measured quantitatively in a peroxidase catalyzed reaction that produces a colour. Absorbance was measured at 500 nm. The color intensity is proportional to cholesterol concentration.

Determination of Triglycerides

Triglycerides were measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H₂O₂, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm (Van Heerden et al., 2020).

Determination of High-density lipoprotein (HDL) cholesterol

In the presence of Mn²⁺ and heparin, chylomicrons, VLDL, and LDL are precipitated, leaving only HDL in solution. The precipitated materials were sedimented by centrifugation, and the HDL containing supernatant can be removed (Li et al., 2019).

Determination of Low-Density-Lipoprotein Cholesterol (LDL-cholesterol)

LDL-cholesterol was calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

$$[\text{LDL-cho}] = [\text{total chol}] - [\text{HDL-cho}] - [\text{TG}]/5$$

where $[\text{TG}]/5$ is an estimate of VLDL-cholesterol and all values were expressed in mg/dL. This was done as described by the method of Fawwad et al., (2016).

Statistical analysis

Data obtained was subjected to Statistical Package for Social Sciences (SPSS) version 25 (IBM, USA, 2018). Lipid profile (total cholesterol, low-density lipoprotein, high-density lipoprotein, triglyceride, and very low-density lipoprotein) and blood glucose level were analyzed using one-way analysis of variance (ANOVA) followed by post Hoc multiple Fishers LSD comparison. Student paired T-tests was used to analyzed body weight. Results were presented in mean and standard deviation and values were considered significant at $p < 0.05$.

Results

Table 1 result showed a significant decrease in the body weight of the rats in group B when compared to group A. However, the body weights in groups C, D, E and F were increased significantly when compared to group B. Table 2 demonstrated that the blood glucose level increased significantly in group B when compared to the control group, however, it was remarkably reversed in the test groups when compared to group B. Table 3 demonstrated that TC and TG levels increased significantly in group B when compared to the control group, and remarkably reduced in groups C, D, E and F when compared to group B. Table 4 demonstrated that HDL decreased significantly, while LDL and VLDL increased remarkably in group B when compared to group A. Conversely, HDL increased significantly, while LDL and VLDL decreased remarkably in groups C, D, E and F when compared to group B.

Table 1: Values of body weight on the study animals

Groups (N=5)	Initial weight (g)	Final weight (g)	P-value	T-value
Group A (control)	145.80±2.45	175.09±3.65	0.012*	-6.423
Group B (60 mg/kg of STZ only)	175.45±4.86	135.81±8.75	0.020*	-7.246
Group C (60 mg/kg of STZ + 50mg/kg of MET)	165.56±10.76	195.45±11.54	0.003*	-5.123
Group D (60 mg/kg of STZ + 25mg/kg of Caffeine)	180.45±7.67	201.26±3.61	0.011*	-4.234
Group E (60 mg/kg of STZ + 50mg/kg of Caffeine)	173.86±4.12	194.67±3.49	0.024*	-8.011

Group F (50 mg/kg of Metformin + 50mg/kg of Caffeine)	160.05±4.12	188.33±3.49	0.004*	-4.020
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Values are presented as mean ± sem.

Table 2: Values of blood glucose on the study animals

Groups (N=5)	Blood glucose level (mg/dL)			
	Day 0	Day 7	Day 14	Day 21
Group A (control)	86.33±2.62	85.67±3.45	82.33±3.74	88.40±2.53
Group B (60 mg/kg of STZ only)	416.67±44.10*	426.00±22.45*	430.78±28.87*	401.00±26.38*
Group C (60 mg/kg of STZ + 50mg/kg of MET)	408.85±10.76*	292.00±21.66*	138.67±11.05*	89.00±8.50*
Group D (60 mg/kg of STZ + 25mg/kg of Caffeine)	395.56±18.42*b	301.00±30.89*b	235.00±14.01*a	126.33±5.78*a
Group E (60 mg/kg of STZ + 50mg/kg of Caffeine)	411.26±14.15*b	291.67±18.78*b	245.00±20.82*a	100.33±4.81*a
Group F (50 mg/kg of Metformin + 50mg/kg of Caffeine)	353.09±44.22*b	277.55±43.00*b	115.50±33.05*a	95.77±2.06*a

Values are presented as mean ± sem.

Table 3: Values of TC and TG on the study animals

Groups (N=5)	Total Cholesterol level (mg/dl)	Triglyceride level (mg/dl)
Group A (control)	231.99±4.32	225.09±9.29
Group B (60 mg/kg of STZ only)	268.96±8.01*	249.25±2.34*
Group C (60 mg/kg of STZ + 50mg/kg of MET)	234.29±5.64*	217.99±7.92*
Group D (60 mg/kg of STZ + 25mg/kg of Caffeine)	244.78±7.81*a	227.88±8.82*a
Group E (60 mg/kg of STZ + 50mg/kg of Caffeine)	240.41±6.55*a	223.57±5.39*b

Group F (50 mg/kg of Metformin + 50mg/kg of Caffeine)	203.05±7.33 ^{*a}	211.00±0.66 ^{*b}
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Values are presented as mean ± sem.

Table 4: Values of HDL, LDL and VLDL on the study animals

Groups (N=)	High density lipoprotein (mg/dl)	Low density lipoprotein (mg/dl)	Very low-density lipoprotein
Group A (control)	178.68±1.55	29.77±2.52	2.98±0.52
Group B (60 mg/kg of STZ only)	140.56±4.30 ^{*b}	86.43±1.09 ^{*b}	7.43±1.09 ^{*b}
Group C (60 mg/kg of STZ + 50mg/kg of MET)	157.98±0.64 ^{*b}	63.90±3.56 ^{*a}	3.90±0.06 ^{*b}
Group D (60 mg/kg of STZ + 25mg/kg of Caffeine)	161.61±3.31 ^{*b}	70.34±2.84 ^{*a}	4.34±0.14 ^{*b}
Group E (60 mg/kg of STZ + 50mg/kg of Caffeine)	150.89±2.84 ^{*b}	52.29±2.86 ^{*a}	5.29±0.16 ^{*a}
Group F (50 mg/kg of Metformin + 50mg/kg of Caffeine)	168.05±0.04 ^{*b}	59.50±2.86 ^{*a}	2.09±1.11 ^{*a}

Values are presented as mean ± sem.

Discussion

Diabetes mellitus has been linked to lipid dysfunction resulting from oxidative stress processes, which is associated with insulin resistance rather than hyperglycemia. However, these changes in the complex metabolic pathway have shown to alter lipoprotein changes and impaired fasting blood glucose and impaired glucose tolerance associated with type II diabetes mellitus (Jialal & Singh, 2019). However, the study investigates the effect of caffeine on the blood glucose levels and lipid profile parameters in streptozotocin-induced diabetic male albino wistar rats.

Hyperglycemia is a condition characterised, which resulted in lipid peroxidation pathway following STZ toxicity. However, DM is a clinical condition that has shown to have several complications associated with elevated blood glucose level, such as dyslipidemia which is disease condition characterised by an abnormal lipid toxicity. The study findings showed that STZ induced diabetes treated group resulted in a significant raised fasting blood glucose level as indicated in group B

when compared to group A, which is the normal control. The physiology following the significant elevation of the blood glucose level is the formation of reactive oxygen species following STZ metabolism in the pancreas, which is linked to production of hydroxyl radicals in the pancreas. However, following its excessive accumulation in the pancreatic beta-cells, it activates the pancreatic catalase, which potentiates further production of hydroxyl radicals dispersing free oxygen radicals that causes destruction of beta cells of the pancreas (Ighodaro & Akinloye, 2018). The study corresponds to the findings of Alsenosy et al. (2019), Nna et al. (2018), Pratiwi et al. (2021), and Fajarwati et al. (2023) demonstrating a significant increase in blood glucose level following STZ induced diabetic rats. Following metformin treatment in diabetic rats as indicated in group C, the study indicated a significant reduction of fasting blood glucose level, which is linked to the activation of the 5'-AMP-activated protein kinase, a key regulator of cellular energy metabolism, which in turn decreases the production of cyclic AMP or acting through the mitochondrial complex I pathway through its suppression (Rena et al., 2017). However, metformin reduces hepatic glucose production, but the gastrointestinal tract plays a crucial role in this effect, particularly through the secretion of the hormone glucagon-like peptide 1 (GLP-1). Bile acids, which regulate glucose metabolism, are involved in fat digestion and absorption. Exposure to bile acids induces GLP-1 secretion, changes gut microbiota composition, and reduces postprandial blood glucose excursions in humans with and without type 2 diabetes (Sansome et al., 2020). The study showed similarity to the report of Atanu et al. (2018), Mahmoudi et al. (2018), and Chukwunonso et al. (2016) reported a significantly declined level in fasting blood glucose level in diabetic rats following metformin intake, which agrees with the study findings. Nahar et al. (2021), Putilin et al. (2020), Shen et al. (2020) showed a significant decline in blood glucose level following metformin intake in STZ diabetic rats, which corroborates the study findings.

Further, treatments with caffeine indicate a significant reduction in the blood glucose level when comparison was made to the diabetic groups. The mechanism following the significant reduction in fasting blood glucose level is enhancing insulin sensitivity and beta-cell function through improved insulin/IGF-1 signalling via induction of insulin receptor substrate 2 (Park et al., 2007). The study findings corroborate the reports of Alshabi et al. (2021), and Othman et al. (2023), demonstrating a significant decrease in the fasting blood glucose level in diabetic rats following caffeine or coffee intake. Xue et al. (2021) indicated a non-significant decrease in the fasting blood glucose level in diabetic rats following STZ induction and treatments with caffeine.

Also, the study indicated a significant increase in the total cholesterol level in diabetic rats following STZ induction, which is linked to dampened effect of glucose uptake by the reduction of glucose transported activity. Further, a proposed mechanism in increased levels of total cholesterol in diabetic rats following STZ is loss of mitochondrial potential as a consequence of cholesterol accumulation in mitochondrial membrane also favors the opening of the transition pores with the release of cytochrome C and the induction of apoptosis (Carrasco-Pozo et al., 2016) following increased lipid peroxidation. Jaishree and Narsimha(2020), and Aktay et al. (2019), reported a significant elevation of total cholesterol level in diabetic rats following STZ induction, which showed similarities to the study findings. However, treatment with metformin showed a significantly decrease in the total cholesterol level following STZ diabetic rats; the physiology linked to this action results from the depressed transcription driven by glucose sensor carbohydrate-responsive element-binding protein (ChREBP), not intracellular cholesterol sensor sterol regulatory element-binding protein 2 (SREBP2). It decreases ChREBP expression and inhibits its transcriptional activity by blocking nuclear translocation, which is attributed to decreased intracellular glucose and glucose metabolite levels, thereby inhibiting its transcriptional activity (Hu et al., 2021). Akintola et al. (2019) reported a significant decline in the total cholesterol following

treatment with metformin in diabetic rats, which aligns with the study findings. The study showed similarities to the reports of Li et al. (2019), Atanu et al. (2018), who revealed a significant decline in the total cholesterol level in STZ induced diabetes. Further, the study findings demonstrated a significant decline in the total cholesterol level in diabetic rats following caffeine intake as indicated in groups D and E. The physiology linked to these changes in the cholesterol level is associated with reduced activities of the key enzymes of lipogenesis, which are acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and/or stearoyl-CoA desaturase (SCD). The ACC and FAS are responsible for the first two steps of de novo lipogenesis, while SCD for the synthesis of monounsaturated fatty acids for fat storage (Proença et al., 2014). Also, a precise mechanism is noticed on the activation of the AMP-activated protein kinase (AMPK), which inhibits ACC and FAS (Sharma et al., 2018). The findings of Salem et al. (2022), and showed a significant decline in the total cholesterol level following caffeine intake in STZ-induced diabetic rats, which corroborates the study findings.

The study findings showed a significant increase in the triglyceride levels (TG) in diabetic treated group alone compared to the normal control as indicated in group B compared to A. The physiology linked to the raised level of TG is associated with a loss of mitochondrial potential as a consequence of cholesterol accumulation in mitochondrial membrane also favors the opening of the transition pores with the release of cytochrome C and the induction of apoptosis (Carrasco-Pozo et al., 2016) following increased lipid peroxidation. However, the study corroborates the findings of Akinola et al. (2019), and Wang et al. (2022) demonstrating a significant increased level of TG in diabetes mellitus. Antwi-Baffour et al. (2018) showed a significant decrease in the TG in diabetic rats compared to non-diabetic rats, which contradict the study findings. Though, treatment with metformin as indicated in group C following diabetes induction revealed a significant decline in the TG level is associated with decreased levels of ChREBP expression and inhibits its transcriptional

activity by blocking nuclear translocation, which is attributed to decreased intracellular glucose and glucose metabolite levels, thereby inhibiting its transcriptional activity (Hu et al., 2021). Abdel-Moneim et al. (2022), reported a significant decline in the TG levels in diabetic model following metformin treatment, which showed similarity to the study outcome. Antwi-Baffour et al. (2018) reported a significant increase TG levels following metformin intake, which contradict the study findings. Also, the study revealed a significant decrease levels of TG in groups D and E following treatments with caffeine, the physiology linked to the decrease levels of TG is associated with the activation of the AMP-activated protein kinase (AMPK), which inhibits ACC and FAS (Sharma et al., 2018), which resulted in down regulation of lipolysis through lipid pathway. The reports of Ilmiawati et al. (2019), revealed a significant decrease in the TG level in diabetic rats following caffeine intake, which showed similarities with the study findings.

Further, the study demonstrated a significant decrease in the HDL levels in diabetic group compared to the non-diabetic group, the physiology linked to the significant decrease in the HDL levels is increased in lipid peroxidation, which resulted in generation of reactive oxygen species. However, the study findings show similarities with the report of Kralova et al. (2021) indicating a significant decrease in the HDL level in diabetes mellitus. However, treatments with metformin showed a significant increase in the level of HDL as indicated in group C. The physiology showed similarity as indicated in the TG discussion above. The study showed similarity to the report of , Li et al. (2019) reveling a significant increase in the HDL level following treatment with metformin. Abbasnezhad et al. (2015), reported “a non-significant increase in the HDL level following metformin intake in STZ diabetic model, refute the study findings”. Also, the study demonstrated a significant decrease in the HDL level following caffeine intake as shown in group D and E, which is linked activation of the AMP-activated protein kinase (AMPK), which inhibits ACC and FAS (Sharma et al., 2018), which resulted in down regulation of lipolysis through lipid pathway. The

study revealed that STZ resulted in a significant increase in the LDL and VLDL level in diabetic control compared to non-diabetic control. The mechanism of action following the significant increase in the LDL levels is associated with reduced activities of the key enzymes of lipogenesis, which are acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and/or stearyl-CoA desaturase (SCD). The study showed similarity with the findings of Kralova et al. (2021), revealing a significant increase in the LDL level following STZ diabetic rats. However, treatment with metformin showed a significant decrease in the LDL levels following STZ diabetic model. The mechanism of action is the same as above as indicated in total cholesterol level in diabetic rats. Zhang et al. (2017), reported “a significant decrease in the LDL and VLDL level in diabetic rats following metformin intake, which corroborates the study findings. Further, it was indicated that a significant decrease was shown by caffeine on the LDL and VLDL levels in diabetic rats. The physiology responsible is the presence of alkaloids, which tend to combat ROS formation and help inhibit the activities of ACC and FAS by the activation of AMPK pathway”. The study corroborates the findings of Salem et al. (2022) demonstrating a significant decrease in the LDL and VLDL following caffeine intake.

The study findings showed that STZ after diabetic induction resulted in a significant body weight loss, which result from the altering of the HPA-axis responsible for satiety control in the hypothalamus. However, STZ is known to be toxic by decreasing the appetite control centers through inhibition of the neuro-fibers sensation regulating feed intake. The study corresponds to report of Gong et al. (2012) revealing a significant weight loss by STZ in diabetic mice, which is line with the study findings. Also, Pratiwi et al. (2021), and Fajarwati et al. (2023) demonstrated a “significant weight loss following STZ diabetic rats, which corroborates the study findings. However, treatments with metformin improved body weight gain significantly following STZ induction. The physiology linked to the significant increase in the body weight is associated with

regulation of adiponectin and enhancement of neurofibers associated with the energy homeostasis". Al Za'abi et al. (2021) reported "a significant increase in the percentage gain weight following the administration of metformin in alloxan-diabetic rats, which is in line with the study findings". Nna et al. (2018) revealed a significant increase in the body weight gain following metformin intake in STZ-induced diabetic rats, which corroborates the study findings.

The study disagrees with the reports of Ali et al. (2019) demonstrated "a non-significant reduction in the body weight following treatment with metformin in STZ diabetic rats". Jiang et al. (2017) reported a significant reduction in the body weight gain following metformin in diabetic rats, which contradict the study findings.

Further, treatment with caffeine showed a significant increase in the body weight in groups D and E, which is linked to increase metabolic rate. Imam-Fulani et al. (2019) showed an improved weight gain following caffeine intake in diabetic rats, which is in line with the study findings, however, it contradicts the study report revealing no significant weight change in the healthy rats. Feyisa et al. (2019), showed a significant decrease in weight following coffee intake in diabetic rats, which contradict the study report. Urzúa et al. (2012) showed "a non-significant weight loss following caffeine treatment with diabetics", which disagree with the study findings.

5.2 Conclusion

The study revealed that streptozotocin showed hyperglycemia and lipid dysfunction in diabetic rats. Caffeine and metformin treatments in diabetic rats revealed improved levels of blood glucose level and lipid profile. However, the study indicated that diabetes mellitus showed a decrease in weight gain, and treatments with metformin and caffeine improved weight. However, the determinant for the significantly improved levels of lipid profile and blood glucose level in this study could be dose-dependent when compared to other previous research based model of animals been employed.

Ethical approval

Ethical approval was obtained from the College of medicine, University of Nigeria, Enugu Campus. Rats handling were in line to the National Institute of Health guidelines for laboratory animal care and use (NIHS 1985, Carbone & Austin, 2016).

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

- 1.
- 2.
- 3.

References

1. Tan, S. Y., Mei Wong, J. L., Sim, Y. J., Wong, S. S., Mohamed Elhassan, S. A., Tan, S. H., Ling Lim, G. P., Rong Tay, N. W., Annan, N. C., Bhattamisra, S. K., & Candasamy, M. (2019). Type 1 and 2 diabetes mellitus: A review on current treatment approach and gene therapy as potential intervention. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 13(1), 364–372. <https://doi.org/10.1016/J.DSX.2018.10.008>
2. Green I. Kinikanwo, Charles C. Nwafor and Weleh I. Iyke (2021). Attenuation of Reproductive Dysfunctions by Hydroethanolic Leaf Extract of *Fleurya aestuans* in Diabetic

- Rats. *Asian Research Journal of Gynaecology and Obstetrics* 6(2): 24-31; Article no.ARJGO.72641. <https://www.researchgate.net/publication/365607700>
3. Ifedi, I. C., Ezeokafor, E. N., Dim, C. N., Dike, C. C., Eyeghre, O. A., Hudu, A. A., & Okoye, K. P. (2021). Hypoglycemic and Hepatocurative activities of Aju-Mbaise on alloxan-diabetic model in male Wistar rats. *International Journal of Science Academic Research*, 02(05), 1515–1520. <http://www.scienceijsar.com>
 4. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., Williams, R., Saeedi, P., Almutairi, R., Montoya, P. A., Basit, A., Phane Besanç, S., Bommer, C., ... Zhang, P. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, 157(107843), 1–10. <https://doi.org/10.1016/J.DIABRES.2019.107843>
 5. Yusuf, S., Rangarajan, S., Teo, K., Islam, S., Li, W., Liu, L., Bo, J., Lou, Q., Lu, F., Liu, T., Yu, L., Zhang, S., Mony, P., Swaminathan, S., Mohan, V., Gupta, R., Kumar, R., Vijayakumar, K., Lear, S., ... Dagenais, G. (2014). Cardiovascular risk and events in 17 low-, middle-, and high-income countries. *The New England Journal of Medicine*, 371(9), 818–827. <https://doi.org/10.1056/NEJMOA1311890>
 6. Tan, B. K. H., & Ong, K. W. (2014). Influence of Dietary Polyphenols on Carbohydrate Metabolism. *Polyphenols in Human Health and Disease*, 1, 95–111. <https://doi.org/10.1016/B978-0-12-398456-2.00009-8>
 7. Akinlade, O. M., Owoyele, B. V., & Soladoye, A. O. (2021). Streptozotocin-induced type 1 and 2 diabetes in rodents: A model for studying diabetic cardiac autonomic neuropathy. *African Health Sciences*, 21(2), 719–727. <https://doi.org/10.4314/ahs.v21i2.30>
 8. Furman, B. L. (2021). Streptozotocin-Induced Diabetic Models in Mice and Rats. *Current Protocols*, 1(4), e78. <https://doi.org/10.1002/CPZ1.78>
 9. Gurung, P., & Jialal, I. (2021). Plasma Glucose. *StatPearls*. <https://www.ncbi.nlm.nih.gov/books/NBK541081/>
 10. Qi, X., & Tester, R. F. (2019a). Fructose, galactose and glucose – In health and disease. *Clinical Nutrition ESPEN*, 33, 18–28. <https://doi.org/10.1016/j.clnesp.2019.07.004>
 11. Dhatariya, K., Corsino, L., & Umpierrez, G. E. (2020). Management of Diabetes and Hyperglycemia in Hospitalized Patients. *Endotext*. <https://www.ncbi.nlm.nih.gov/books/NBK279093/>
 12. Yau, M., Maclaren, N. K., & Sperling, M. A. (2021). Etiology and Pathogenesis of Diabetes Mellitus in Children and Adolescents. *Endotext*. <https://www.ncbi.nlm.nih.gov/books/NBK498653/>
 13. Christensen, A. A., & Gannon, M. (2019). The Beta Cell in Type 2 Diabetes. *Current Diabetes Reports*, 19(9), 81. <https://doi.org/10.1007/s11892-019-1196-4>
 14. Galicia-garcia, U., Benito-vicente, A., Jebari, S., & Larrea-sebal, A. (2020). Pathophysiology of Type 2 Diabetes Mellitus. *International Journal of Molecular Sciences*, 21(6275), 1–34.
 15. Charles C. Nwafor , Osakwe F. Uchechwukwu , Mfonido G. Umanah , Ekikereabasi P. Inyang and Peters K. Dell (2022): Effect of Hydroethanol Leaf Extract of Fleurya Aestuans

- on Histological and Renal Function Test of an Alloxan Induced Diabetic Wistar Rats . International Journal of Advances in Nephrology Research. 5(1): 56-63, 2022; Article no.IJANR.87887. : <https://www.researchgate.net/publication/365374283>
16. Tao, Z., & Cheng, Z. (2023). Hormonal regulation of metabolism—recent lessons learned from insulin and estrogen. *Clinical Science*, 137(6), 415–434. <https://doi.org/10.1042/CS20210519>
 17. Dasgupta, A., & Wahed, A. (2021). Lipid Metabolism and Disorders. *Clinical Chemistry, Immunology and Laboratory Quality Control*, 105–126. <https://doi.org/10.1016/B978-0-12-815960-6.00010-8>
 18. Lee, Y., & Siddiqui, W. J. (2022). Cholesterol Levels. In: *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan, Idl, 5–12.* <https://www.ncbi.nlm.nih.gov/books/NBK542294/>
 19. Haile, K., & Timerga, A. (2020). Dyslipidemia and Its Associated Risk Factors Among Adult Type-2 Diabetic Patients at Jimma University Medical Center, Jimma, Southwest Ethiopia. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 13, 4589–4597. <https://doi.org/10.2147/DMSO.S283171>
 20. Khatana, C., Saini, N. K., Chakrabarti, S., Saini, V., Sharma, A., Saini, R. V., & Saini, A. K. (2020). Mechanistic Insights into the Oxidized Low-Density Lipoprotein-Induced Atherosclerosis. *Oxidative Medicine and Cellular Longevity*, 2020, 5245308. <https://doi.org/10.1155/2020/5245308>
 21. Blaise, J. H., Park, J. E., Bellas, N. J., Gitchell, T. M., & Phan, V. (2018). Caffeine consumption disrupts hippocampal long-term potentiation in freely behaving rats. *Physiological Reports*, 6(5), e13632. <https://doi.org/10.14814/PHY2.13632>
 22. Arthur Nwafor Chuemere, Ilochi Ogadinma and Nwafor Charles (2018). Evaluation of Blood Glucose-regulating Potential of Honey, Coffee and Vitamin E in Normoglycaemic Male Wistar Rats. *Asian Journal of Research in Medical and Pharmaceutical Sciences* 4(4): 1-7; Article no.AJRIMPS.44133 ISSN: 2457-0745. <https://www.researchgate.net/publication/327641242>
 23. Sanchez-Rangel, E., & Inzucchi, S. E. (2017). Metformin: clinical use in type 2 diabetes. *Diabetologia*, 60(9), 1586–1593. <https://doi.org/10.1007/S00125-017-4336-X>
 24. Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. *Physiological Reviews*, 98(4), 2133–2223. <https://doi.org/10.1152/PHYSREV.00063.2017/ASSET/IMAGES/LARGE/Z9J0041828680019.JPEG>
 25. Nasri, H., & Rafieian-Kopaei, M. (2014). Metformin: Current knowledge. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*, 19(7), 658–664. [/pmc/articles/PMC4214027/](https://pubmed.ncbi.nlm.nih.gov/24214027/)
 26. Morakinyo, A. O., Adekunbi, D. A., Dada, K. A., & Adegoke, O. A. (2013). Coffee consumption attenuates insulin resistance and glucose intolerance in rats fed on high-sucrose diet. *Nigerian Journal of Physiological Sciences*, 28(2), 179–185.
 27. Akash, M. S. H., Rehman, K., & Chen, S. (2014). Effects of coffee on type 2 diabetes mellitus. *Nutrition (Burbank, Los Angeles County, Calif.)*, 30(7–8), 755–763. <https://doi.org/10.1016/J.NUT.2013.11.020>

28. Kempf, K., Herder, C., Erlund, I., Kolb, H., Martin, S., Carstensen, M., Koenig, W., Sundvall, J., Bidel, S., Kuha, S., & Tuomilehto, J. (2010). Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial. *The American Journal of Clinical Nutrition*, 91(4), 950–957. <https://doi.org/10.3945/AJCN.2009.28548>
29. Akinola, F. F., Ogundiran, S. ., Oladapo, A. A., & Busari, A. O. (2019). Effect Of Caffeinated Drink On Lipid Profile In Alloxan-Induced Diabetic Male Albino Rats. *International Journal of Advanced Research*, 7(8), 258–265. <https://doi.org/10.21474/IJAR01/9498>
30. Feyisa, T. O., Melka, D. S., Menon, M., Labisso, W. L., & Habte, M. L. (2019). Investigation of the effect of coffee on body weight, serum glucose, uric acid and lipid profile levels in male albino Wistar rats feeding on high-fructose diet. *Laboratory Animal Research*, 35(1), 29. <https://doi.org/10.1186/S42826-019-0024-Y>
31. Meng, X. M., Ma, X. X., Tian, Y. L., Jiang, Q., Wang, L. L., Shi, R., Ding, L., & Pang, S. G. (2017). Metformin improves the glucose and lipid metabolism via influencing the level of serum total bile acids in rats with streptozotocin-induced type 2 diabetes mellitus. *European Review for Medical and Pharmacological Sciences*, 21(9), 2232–2237.
32. Anurag, P., & Anuradha, C. V. (2002). Metformin improves lipid metabolism and attenuates lipid peroxidation in high fructose-fed rats. *Diabetes, Obesity & Metabolism*, 4(1), 36–42. <https://doi.org/10.1046/J.1463-1326.2002.00178.X>
33. National Institute of Health. Laboratory Animal Welfare. United State Department of Health and Human Services. 1985;14:8.
34. Carbone, L., & Austin, J. (2016). Pain and laboratory animals: Publication practices for better data reproducibility and better animal welfare. *PLoS ONE*, 11(5), e0155001. <https://doi.org/10.1371/journal.pone.0155001>
35. Charles C. N., Ovie F. O., Oliver N. L. and Onyewuchi M. O. (2021). Haemato-Protective Efficacies Of Hydroethanolic Extract Of Fleurya Aestuans Leaves Against Alloxan. *World Journal Of Pharmacy And Pharmaceutical Sciences*. 10(11), 23-34. <https://www.researchgate.net/publication/365607679>
36. Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54(4), 275–287. <https://doi.org/10.1007/BF01234480>
37. Parasuraman, S., Raveendran, R., & Kesavan, R. (2010). Blood sample collection in small laboratory animals. *Journal of Pharmacology and Pharmacotherapeutics*, 1(2), 87–93. <https://doi.org/10.4103/0976-500X.72350>
38. Charles C. N, Amah-Tariah F. S. and Dapper D. V (2021): Effect of Hydroethanolic Extract of FleuryaAestuans on Haematological Parameters and Oxidative Indices of Phenylhydrazin Induced Toxicity. *International Journal of Research and Reports in Hematology*. 4(3):17 27. Article no.IJR2H.72627. <https://www.researchgate.net/publication/365374238>
39. Li, L. H., Dutkiewicz, E. P., Huang, Y. C., Zhou, H. B., & Hsu, C. C. (2019). Analytical methods for cholesterol quantification. *Journal of Food and Drug Analysis*, 27(2), 375–386. <https://doi.org/10.1016/J.JFDA.2018.09.001>
40. Van Heerden, C., Magwete, A., & Mabuza, D. (2020). Evaluating the need for free glycerol

- blanking for serum triglyceride measurements at Charlotte Maxeke Johannesburg Academic Hospital. *Clinical Chemistry and Laboratory Medicine*, 58(8), 1257–1264. <https://doi.org/10.1515/CCLM-2019-0991/MACHINEREADABLECITATION/RIS>
41. Fawwad, A., Sabir, R., Riaz, M., & Moin, H. (2016). Measured versus calculated LDL-cholesterol in subjects with type 2 diabetes. *Pakistan Journal of Medical Sciences*, 32(4), 955–960. <https://doi.org/10.12669/PJMS.324.9896>
 42. Jialal, I., & Singh, G. (2019). Management of diabetic dyslipidemia: An update. *World Journal of Diabetes*, 10(5), 280–290. <https://doi.org/10.4239/wjd.v10.i5.280>
 43. Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, 54(4), 287–293. <https://doi.org/10.1016/J.AJME.2017.09.001>
 44. Alsenosy, A. W. A., El-Far, A. H., Sadek, K. M., Ibrahim, S. A., Atta, M. S., Sayed-Ahmed, A., Al Jaouni, S. K., & Mousa, S. A. (2019). Graviola (*Annona muricata*) attenuates behavioural alterations and testicular oxidative stress induced by streptozotocin in diabetic rats. *PLoS ONE*, 14(9). <https://doi.org/10.1371/journal.pone.0222410>
 45. Nna, V. U., Abu Bakar, A. B., Md Lazin, M. R. M. L., & Mohamed, M. (2018). Antioxidant, anti-inflammatory and synergistic anti-hyperglycemic effects of Malaysian propolis and metformin in streptozotocin-induced diabetic rats. *Food and Chemical Toxicology*, 120, 305–320. <https://doi.org/https://doi.org/10.1016/j.fct.2018.07.028>
 46. Pratiwi, R. Y., Elya, B., Setiawan, H., Solawati, A., & Rosmalena. (2021). Alterations in Body Weight, Blood Glucose Levels, and Lipid Profiles in High-Fat Diet-Low Dose Streptozotocin-Induced Diabetic Rats. *Pharmacognosy Journal*, 13(6s), 1562–1567.
 47. Fajarwati, I., Solihin, D. D., Wresdiyati, T., & Batubara, I. (2023). Administration of alloxan and streptozotocin in Sprague Dawley rats and the challenges in producing diabetes model. *IOP Conference Series: Earth and Environmental Science*, 1174(1), 1–13. <https://doi.org/10.1088/1755-1315/1174/1/012035>
 48. Rena, G., Hardie, D. G., & Pearson, E. R. (2017). The mechanisms of action of metformin. *Diabetologia*, 60(9), 1577–1585. <https://doi.org/10.1007/S00125-017-4342-Z>
 49. Sansome, D. J., Xie, C., Veedfald, S., Horowitz, M., Rayner, C. K., & Wu, T. (2020). Mechanism of glucose-lowering by metformin in type 2 diabetes: Role of bile acids. *Diabetes, Obesity & Metabolism*, 22(2), 141–148. <https://doi.org/10.1111/DOM.13869>
 50. Atanu, F. O., Avwioroko, O. J., & Momoh, S. (2018). Anti-diabetic effect of combined treatment with Aloe vera gel and Metformin on alloxan-induced diabetic rats. *Journal of Ayurvedic and Herbal Medicine*, 4(1), 1–5.
 51. Mahmoudi, Y., Gholami, M., Nikbakht, H., & Ebrahim, K. (2018). Effect of High Intensity Interval Training with Metformin on Lipid Profiles and HbA1c in Diabetic Rats. *Iranian Journal of Diabetes and Obesity*, 10(3), 144–150.
 52. Chukwunonso Obi, B., Chinwuba Okoye, T., Okpashi, V. E., Nonye Igwe, C., & Olisah Alumanah, E. (2016). Comparative study of the antioxidant effects of metformin, glibenclamide, and repaglinide in alloxan-induced diabetic rats. *Journal of Diabetes Research*, 2016, 1–6. <https://doi.org/10.1155/2016/1635361>
 53. Nahar, N., Mohamed, S., Mustapha, N. M., Lau, S., Ishak, N. I. M., & Umran, N. S. (2021).

Metformin attenuated histopathological ocular deteriorations in a streptozotocin-induced hyperglycemic rat model. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 394(3), 457–467. <https://doi.org/10.1007/s00210-020-01989-w>

54. Putilin, D. A., Evchenko, S. Y., Fedoniuk, L. Y., Tokarsky, O. S., Kamyshny, O. M., Migenko, L. M., Andreychyn, S. M., Hanberher, I. I., & Bezruk, T. O. (2020). The Influence of Metformin to the Transcriptional Activity of the mTOR and FOX3 Genes in Parapancreatic Adipose Tissue of Streptozotocin-Induced Diabetic Rats. *Journal of Medicine and Life*, 13(1), 50–55. <https://doi.org/10.25122/JML-2020-0029>
55. Shen, X., Wang, L., Zhou, N., Gai, S., Liu, X., & Zhang, S. (2020). Beneficial effects of combination therapy of phloretin and metformin in streptozotocin-induced diabetic rats and improved insulin sensitivity in vitro. *Food & Function*, 11(1), 392–403. <https://doi.org/10.1039/C9FO01326A>
56. Park, S., Jang, J. S., & Hong, S. M. (2007). Long-term consumption of caffeine improves glucose homeostasis by enhancing insulinotropic action through islet insulin/insulin-like growth factor 1 signaling in diabetic rats. *Metabolism: Clinical and Experimental*, 56(5), 599–607. <https://doi.org/10.1016/J.METABOL.2006.12.004>
57. Alshabi, A. M., Alkahtani, S. A., Shaikh, I. A., & Habeeb, M. S. (2021). Caffeine modulates pharmacokinetic and pharmacodynamic profiles of pioglitazone in diabetic rats: Impact on therapeutics. *Saudi Medical Journal*, 42(2), 151–160. <https://doi.org/10.15537/SMJ.2021.2.25695>
58. Othman, M. A., Fadel, R., Tayem, Y., Jaradat, A., Rashid, A., Fatima, A., Al-Mahameed, A. E., & Nasr El-Din, W. A. (2023). Caffeine protects against hippocampal alterations in type 2 diabetic rats via modulation of gliosis, inflammation and apoptosis. *Cell and Tissue Research*, 392(2), 443–466. <https://doi.org/10.1007/s00441-022-03735-5>
59. Xue, J., Liu, Y., Zhang, S., Ding, L., Shen, B., Shao, Y., & Wei, Z. (2021). Caffeine improves bladder function in diabetic rats via a neuroprotective effect. *Exp Ther Med*, 21(5), 501. <https://doi.org/10.3892/etm.2021.9932>
60. Carrasco-Pozo, C., Tan, K. N., Reyes-Farias, M., De La Jara, N., Ngo, S. T., Garcia-Diaz, D. F., Llanos, P., Cires, M. J., & Borges, K. (2016). The deleterious effect of cholesterol and protection by quercetin on mitochondrial bioenergetics of pancreatic β -cells, glycemic control and inflammation: In vitro and in vivo studies. *Redox Biology*, 9, 229–243. <https://doi.org/10.1016/J.REDOX.2016.08.007>
61. Jaishree, V., & Narsimha, S. (2020). Swertiamarin and quercetin combination ameliorates hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced type 2 diabetes mellitus in wistar rats. *Biomedicine & Pharmacotherapy*, 130, 110561. <https://doi.org/https://doi.org/10.1016/j.biopha.2020.110561>
62. Aktay, G., Gürsoy, Ş. Ö., Uyumlu, U., Ünüvar, S., & İlhan, N. (2019). Protective effect of atorvastatin on oxidative stress in streptozotocin-induced diabetic rats independently their lipid-lowering effects. *Journal of Biochemical and Molecular Toxicology*, 33(5), e22295. <https://doi.org/https://doi.org/10.1002/jbt.22295>
63. Hu, Y., Ding, B., Shen, Y., Yan, R. N., Li, F. F., Sun, R., Jing, T., Lee, K. O., & Ma, J. H. (2021). Rapid Changes in Serum Testosterone in Men With Newly Diagnosed Type 2 Diabetes With Intensive Insulin and Metformin. *Diabetes Care*, 44(4), 1059–1061.

<https://doi.org/10.2337/DC20-1558>

64. Sharma, L., Lone, N. A., Knott, R. M., Hassan, A., & Abdullah, T. (2018). Trigonelline prevents high cholesterol and high fat diet induced hepatic lipid accumulation and lipotoxicity in C57BL/6J mice, via restoration of hepatic autophagy. *Food and Chemical Toxicology*, *121*, 283–296. <https://doi.org/10.1016/j.fct.2018.09.011>
65. Salem, I. S., Mageed, N. M. A., & Rahman, H. S. F. A. (2022). Effect Of Green Coffee Bean Extract On Rats Suffering From Diabetes. *Journal of Pharmaceutical Negative Results*, *13*, 8130–8143. <https://doi.org/10.47750/PNR.2022.13.S09.950>
66. Wang, L., Yan, N., Zhang, M., Pan, R., Dang, Y., & Niu, Y. (2022). The association between blood glucose levels and lipids or lipid ratios in type 2 diabetes patients: A cross-sectional study. In *Frontiers in Endocrinology* (Vol. 13, p. 969080). <https://www.frontiersin.org/articles/10.3389/fendo.2022.969080>
67. Antwi-Baffour, S., Kyeremeh, R., Boateng, S. O., Annison, L., & Seidu, M. A. (2018). Haematological parameters and lipid profile abnormalities among patients with Type-2 diabetes mellitus in Ghana. *Lipids in Health and Disease*, *17*(1), 283. <https://doi.org/10.1186/s12944-018-0926-y>
68. Abdel-Moneim, A.-M. H., Lutfi, M. F., Alsharidah, A. S., Shaker, G., Faisal, W., Abdellatif, A. A. H., Rugaie, O. A., Mohany, K. M., Eid, S. Y., El-Readi, M. Z., & Alsharidah, M. (2022). Short-Term Treatment of Metformin and Glipizide on Oxidative Stress, Lipid Profile and Renal Function in a Rat Model with Diabetes Mellitus. In *Applied Sciences* (Vol. 12, Issue 4, p. 2019). <https://doi.org/10.3390/app12042019>
69. Ilmiawati, C., Fitri, F., Rofinda, Z. D., & Reza, M. (2020). Green coffee extract modifies body weight, serum lipids and TNF- α in high-fat diet-induced obese rats. *BMC Research Notes*, *13*(1), 208. <https://doi.org/10.1186/s13104-020-05052-y>
70. Kralova, E., Marusakova, M., Hadova, K., Krenek, P., & Klimas, J. (2021). Dapagliflozin elevates plasma high-density lipoprotein levels and influences visceral fat gene expression in streptozotocin-induced diabetes mellitus. *Journal of Pharmacy and Pharmacology*, *73*(6), 778–784. <https://doi.org/10.1093/jpp/rgab005>
71. Abbasnezhad, A., Hayatdavoudi, P., Niazmand, S., & Mahmoudabady, M. (2015). The effects of hydroalcoholic extract of *Nigella sativa* seed on oxidative stress in hippocampus of STZ-induced diabetic rats. *Avicenna Journal of Phytomedicine*, *5*(4), 333–340. <https://doi.org/10.22038/ajp.2015.3921>
72. Zhang, X., Imperatore, G., Thomas, W., Cheng, Y., Lobelo, F., Norris, K., HM, D., MK, A., Gruss, S., Bardenheier, B., Cho, P., Garcia de Quevedo, I., Mudaliar, U., Saaddine, J., Geiss, L., & Gregg, E. (2017). Effect of lifestyle interventions on glucose regulation among adults without impaired glucose tolerance or diabetes: A systematic review and meta-analysis. *Diabetes Research and Clinical Practice*, *123*, 149–164. <https://doi.org/10.1016/J.DIABRES.2016.11.020>
73. Gong, L., Goswami, S., Giacomini, K. M., Altman, R. B., & Klein, T. E. (2012). Metformin pathways: Pharmacokinetics and pharmacodynamics. *Pharmacogenetics and Genomics*, *22*(11), 820–827. <https://doi.org/10.1097/FPC.0B013E3283559B22>
74. Al Za'Abi, M., Ali, B. H., Al Suleimani, Y., Adham, S. A., Ali, H., Manoj, P., Ashique, M., & Nemmar, A. (2021). The effect of metformin in diabetic and non-diabetic rats with

- experimentally-induced chronic kidney disease. *Biomolecules*, 11(6), 84. <https://doi.org/10.3390/biom11060814>
75. Ali, O. (2019). Genetics of type 2 diabetes. *World Journal of Diabetes*, 4(4), 114–123. <https://doi.org/10.4239/WJD.V4.I4.114>
76. Jiang, G. J., Han, X., Tao, Y. L., Deng, Y. P., Yu, J. W., Cai, J., Ren, G. F., & Sun, Y. N. (2017). Metformin ameliorates insulinitis in STZ-induced diabetic mice. *PeerJ*, 2017(4), 1–13. <https://doi.org/10.7717/peerj.3155>
77. Imam-Fulani, A. O., Atta, A. O., & Owoyele, B. V. (2019). Effects of Caffeine Administration on Body Weight and Memory in Healthy and Streptozotocine-Induced Diabetic Female Wistar Rats. *Tropical Journal of Health Sciences*, 26(1), 1–7. <https://doi.org/10.4314/TJHC.V26I1>
78. Urzúa, Z., Trujillo, X., Huerta, M., Trujillo-Hernández, B., Ríos-Silva, M., Onetti, C., Ortiz-Mesina, M., & Sánchez-Pastor, E. (2012). Effects of chronic caffeine administration on blood glucose levels and on glucose tolerance in healthy and diabetic rats. *Journal of International Medical Research*, 40(6), 2220–2230. https://doi.org/10.1177/030006051204000620/ASSET/IMAGES/LARGE/10.1177_030006051204000620-FIG5.JPEG