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Metabolic and structural hepatic changes in diabetes and intracerebral haemorrhage: Comparative study of lipid-lowering and antidiabetic drugs

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Type 2 diabetes is a major global health problem frequently accompanied by hepatic metabolic disturbances. An experimental model of streptozotocin-induced type 2 diabetes complicated by intracerebral haemorrhage was established in rats. Pharmacological correction was performed using rosuvastatin, metformin, or vitamin D. Liver morphology was assessed by histological examination, while lipid and biochemical parameters were evaluated using spectrometric methods. Streptozotocin-induced type 2 diabetes was associated with significant dysregulation of glucose metabolism, hepatic cholesterol and triglyceride levels, and aminotransferase activity. The addition of intracerebral haemorrhage resulted in pronounced macrovesicular steatosis, deterioration of the hepatic lipid profile, diapedesis haemorrhage, perivascular oedema, and marked lymphocytic infiltration. Rosuvastatin demonstrated the most pronounced corrective effect, leading to near-complete regression of steatosis, normalisation of lipid parameters, prevention of necrotic and haemorrhagic changes, and restoration of lobular liver architecture. Metformin and vitamin D produced moderate improvements in hepatic morphology and lipid metabolism without full normalisation. Intracerebral haemorrhage markedly aggravates hepatic morphofunctional disturbances in streptozotocin-induced type 2 diabetes, promoting the development of macrovesicular steatosis. Among the agents investigated, rosuvastatin exhibited the greatest hepatoprotective potential, whereas metformin and vitamin D showed moderate corrective effects. These findings support differentiated pharmacological approaches and suggest potential benefits of combination therapy in diabetic hepatopathy. From a research perspective, further work should focus on identifying effective combinations of the drugs studied in this experimental model.

Keywords: STZ-induced type 2 diabetes; liver injury; macrovesicular steatosis; rosuvastatin; metformin; vitamin D.

Introduction

Type 2 diabetes (T2D) remains a major global health challenge, characterised by a steadily increasing prevalence and a high burden of chronic complications (Sulaieva et al., 2024; Steinberg et al., 2025). Beyond classical vascular and renal manifestations, the liver plays a leading role in the pathogenesis of type 2 diabetes due to its key involvement in glucose and lipid metabolism (Dall'Agnese et al., 2022; Lee et al., 2023). Hepatic insulin resistance is now recognised as a pivotal mechanism driving hyperglycaemia, dyslipidaemia, steatotic liver disease, and steatohepatitis (Steinberg et al., 2025), and the progression of metabolic liver disease in type 2 diabetes (Dyomshyna & Reziapov, 2021). The liver is sensitive to insulin signalling owing to the exceptionally high density of insulin receptors on hepatocytes (Frias et al., 2019). Impairment of hepatic insulin responsiveness leads to excessive gluconeogenesis, dysregulated lipid handling, and triglyceride accumulation in hepatocytes, resulting in steatosis and steatohepatitis (Burtis et al., 2012; Li et al., 2022). Experimental evidence indicates that hepatic gluconeogenesis in type 2 diabetes may increase severalfold, highlighting the liver as a critical therapeutic target (Dyomshyna & Reziapov, 2021; Sakurai et al., 2021; Massimino et al., 2023).

Streptozotocin (STZ)-induced models of type 2 diabetes are used to reproduce insulin resistance, hyperglycaemia, and metabolic dysregulation in experimental animals (Etuk, 2010; Ghasemi et al., 2014; Furman, 2021). While hepatic steatosis has been extensively described in STZ-induced type 2 diabetes, the impact of additional systemic stressors, such as intracerebral haemorrhage, on diabetic liver pathology remains insufficiently explored (Nagata & Einama, 2020). Intracerebral haemorrhage induces hypoxia, microcirculatory disturbances, oxidative stress, and inflammatory activation, which may synergistically exacerbate diabetes-associated hepatic injury (MacLellan et al., 2010). The combined effects of type 2 diabetes and intracerebral haemorrhage on liver structure and lipid metabolism are a clinically relevant but understudied pathology. Pharmacological strategies used in type 2 diabetes differ markedly in hepatic effects. Metformin, a first-line antidiabetic agent, primarily improves hepatic insulin sensitivity and suppresses gluconeogenesis via activation of AMP-activated protein kinase (Dziubak et al., 2018; Frias et al., 2019; Wang et al., 2022). One of the most significant outcomes is a marked reduction in cardiovascular mortality among patients with type 2 diabetes (El Messaoudi et al., 2011; Zhyliuk et al., 2021a, 2021b). Statins, including rosuvastatin (Heeba & Hamza, 2015; Fahmy et al., 2022), are widely prescribed to correct diabetic dyslipidaemia (Zhang et al., 2018; Grievink et al., 2019) and exhibit pleiotropic anti-inflammatory and cytoprotective effects (Christiansen et al., 2021; Pfisterer et al., 2024; Kim et al., 2025); however, their impact on the diabetic liver under conditions of combined metabolic and ischemic stress remains controversial. Vitamin D ($1,25(OH)_2D_3$) has emerged as a modulator of lipid metabolism, inflammation (Ning et al., 2015), and oxidative stress in metabolic diseases (Marek et al., 2022; Ravaioli et al., 2022; Du et al., 2023); its role in diabetic liver injury complicated by intracerebral haemorrhage is poorly defined.

To date, no experimental studies have comprehensively evaluated the effects of rosuvastatin, metformin, and vitamin D on hepatic lipid metabolism and morphology in a model of STZ-induced type 2 diabetes complicated by intracerebral haemorrhage. Therefore, the present study highlights the metabolic and structural hepatic changes in the liver of rats under a combined pathological model and compares the corrective effects of rosuvastatin, metformin, and vitamin D under systemic hypoxic stress.

Materials and methods

This study was conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and complied with Directive 2010/63/EU of the European Parliament and of

the Council of 22 September 2010. The study protocol was approved by the Local Animal Ethics Committee of the Faculty of Biology and Ecology, Oles Honchar Dnipro National University. Animals were maintained in standard vivarium conditions at the State Institution "Dnipro Medical University of the Ministry of Health of Ukraine" on a standard diet and with water available ad libitum, at an ambient temperature of 22 ± 2°C, with alternating light/dark cycles in accordance with sanitary and hygienic regulations. The experiment was conducted on sexually mature male Wistar rats weighing 180–230 g, obtained from the Animal Centre "Dali 2011" (Kyiv, Ukraine). All animals were randomly assigned to experimental groups, which minimises the risk of bias. In each experimental group, 6 animals.

Animals were divided into six groups (n = 6 per group):

1. Control group – intact animals receiving physiological saline (5 mL/kg).

2. STZ-induced T2D group – animals with STZ-induced type 2 diabetes receiving physiological saline (5 mL/kg).

3. STZ-induced T2D+Intracerebral haemorrhage group – animals with STZ-induced type 2 diabetes complicated by intracerebral haemorrhage.

4. STZ-induced T2D+Intracerebral haemorrhage+Rosuvastatin group – animals with STZ-induced type 2 diabetes complicated by intracerebral haemorrhage, treated with rosuvastatin (15 mg/kg).

5. STZ-induced T2D+Intracerebral haemorrhage+Metformin group – animals with STZ-induced type 2 diabetes complicated by intracerebral haemorrhage, treated with metformin (250 mg/kg).

6. STZ-induced T2D+Intracerebral haemorrhage+1,25$(OH)_2D_3$ group – animals with STZ induced type 2 diabetes complicated by intracerebral haemorrhage, treated with Vitamin D (500 IU/kg).

An essential stage in the study of diseases is the development of experimental models. Streptozotocin (STZ) is the most widely used diabetogenic compound for modelling experimental diabetes in laboratory animals (Ghasemi et al., 2014). STZ is a synthetic compound – an N-nitroso derivative of glucosamine – originally isolated from *Streptomyces achromogenes*. STZ is toxic to pancreatic cells because it selectively enters pancreatic β cells via the glucose transporter isoform 2 (GLUT2). The pancreato-toxicity of STZ is associated with the alkylating activity of its methyl group, which leads to fragmentation of β-cell DNA (Etuk, 2010).

Administration of STZ in laboratory animals induces pathological changes of varying severity, including peripheral insulin resistance and partial impairment of responses, and widespread β-cell necrosis in the pancreas. The effects of experimental pathology vary with dosage, route of administration, species, strain, age, diet, and other animal-specific factors (Ghasemi et al., 2014).

After intravenous administration, the concentration of STZ in the cerebrospinal fluid approaches the concentration in plasma after 2 hours. STZ accumulates in the liver and kidneys, undergoes hepatic metabolism, and is renally excreted (up to 20% of the administered dose) in unchanged form along with at least three identified metabolites, including methyl nitrosourea. Less than 1% is excreted in faeces, with significant elimination via respiration (Kishore et al., 2017). The impact of STZ on the liver is generally minimal, and hepatotoxic effects are rarely observed. Nevertheless, STZ is contraindicated in cases of impaired hepatic function (Etuk, 2010).

To reproduce an experimental equivalent of type 2 diabetes, animals underwent 24-hour food deprivation (with free access to water), followed by a single intraperitoneal injection of nicotinamide (Sigma Aldrich, USA) at 230 mg/kg body weight. Fifteen minutes later, streptozotocin (Sigma Aldrich, USA) was administered at 65 mg/kg as a 5% solution in citrate buffer (pH 4.5) (Furman, 2021). Blood glucose concentration was measured 72 hours after injections. Animals with glucose levels < 8.3 mmol/L were excluded from the study.

Intracerebral haemorrhage was induced on day 60 after nicotinamide/streptozotocin administration by microinjection of 1 μL sterile physiological saline containing 0.2 IU bacterial collagenase (type IV S, Sigma Aldrich; 1.0 μL, 0.2 IU/μL) through a 26G needle over >5 minutes, following the protocol (MacLellan et al., 2010). Intracerebral haemorrhage is used as a model of systemic hypoxia/haemorrhagic stress. Whereafter, rats received intragastric administration of rosuvastatin (15 mg/kg) (Astrapharm, Vyshneve, Kyiv region, Ukraine) or metformin (250 mg/kg) (Astrapharm, Vyshneve, Kyiv region, Ukraine), or Vitamin D (1,25$(OH)_2D_3$, 500 IU/kg) (Astrapharm, Vyshneve, Kyiv region, Ukraine) for 20 consecutive days.

At the end of the experiment, animals were weighed and euthanised under light anaesthesia (thiopental, 60 μg/kg). The liver was excised, rinsed in physiological saline, and prepared for subsequent analyses. The cytosolic fraction of the liver was obtained by differential centrifugation in homogenization medium containing 250 mM sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4, at 0–3 °C (Wieckowski et al., 2009). The cytosolic fraction was used to determine total hepatic cholesterol and triglyceride levels in the experimental groups.

Total cholesterol, triglycerides, and aminotransferase activity were determined using standard laboratory kits (Cormay, Lomianki, Poland) per the manufacturer's protocol (Burtis et al., 2012). Measurements of experimental samples were conducted using a semi-automated biochemical analyser BS 3000M (SINNOWA Medical Science & Technology Co., Ltd., Nanjing, China, 2018).

Fresh liver tissue was rinsed in physiological saline and subsequently fixed in 10% buffered formalin. Tissues were dehydrated, paraffin-embedded, sectioned at 3 μm using an automatic ultramicrotome (UMTP-6M; SELMI, Sumy, Ukraine, 2017), and stained with haematoxylin and eosin. A researcher blinded to the animals' group affiliation assessed the results, reducing the risk of bias.

Data were analysed using one-way analysis of variance (ANOVA) to compare group means. Before ANOVA, assumptions of normality and homogeneity of variances were assessed. When significant differences were detected, post hoc multiple comparisons were performed using Tukey's honestly significant difference (HSD) test to adjust for multiple testing. Results are expressed as mean (x) ± standard deviation (SD), with sample size (n) indicated in figure legends. Statistical significance was conferred with P-values < 0.05.

Results

Liver injury induced by diabetes and intracerebral haemorrhage led to the hepatic pathology visualised by haematoxylin-eosin staining (Fig. 1). In the control group (Fig. 1a), hepatocytes exhibited well-defined boundaries, uniform cytoplasmic granularity, and preserved nuclei. Vessels displayed normal lumina, with moderately dilated sinusoids. No lipid vacuoles were observed. Inflammatory infiltrates, necrosis, fibrosis, and haemorrhage were absent. The lobular architecture was preserved, with clearly visible central veins and radial arrangement of hepatocytes.

In the STZ-induced type 2 diabetes group (Fig. 1b), moderate microvesicular steatosis was evident, characterised by small cytoplasmic vacuoles within hepatocytes. Partial destruction of the trabecular structure was noted, with hepatocytes losing their orderly arrangement. Moderate sinusoidal dilatation and focal lymphocytic inflammatory infiltrates were observed. No massive necrosis was present, although early dystrophic changes were visible. Imaging features minimal fibrosis, perisinusoidal.

In the STZ-induced type 2 diabetes group complicated by intracerebral haemorrhage (Fig. 1c), pronounced degenerative and necrotic changes were observed. Hepatocyte cytoplasm appeared lighter, with indistinct cell boundaries. Macrovesicular steatosis was evident, with large vacuoles displacing nuclei toward the periphery. Haemorrhagic foci, characterised by extravascular erythrocytes (diapedesis haemorrhages), were clearly visible in the parenchyma. Signs of perivascular oedema were present. Inflammatory infiltrates were extensive, occasionally accompanied by leukocytic destruction. Fibrous septa between lobules indicated stromal activation (early periportal fibrosis). Lobular architecture was markedly disrupted, with indistinct trabecular boundaries and deformed central veins.

Morphological analysis of the rat liver in STZ-induced type 2 diabetes complicated by intracerebral haemorrhage under the influence of statins, antidiabetic agents, and vitamin preparations (Fig. 2). In the livers of rats with STZ-induced type 2 diabetes complicated by intracerebral haemorrhage treated with rosuvastatin (Fig. 2a), lipid vacuoles were observed in hepatocytes of peripheral lobular regions, indi-

cating residual (moderate) microvesicular steatosis. No clear signs of inflammation were detected. Necrosis and fibrosis were absent. Haemorrhagic foci were not visualised. Lobular architecture was preserved, although moderate disorganisation of hepatocyte trabeculae was noted. Central veins and sinusoids showed no marked pathological changes.

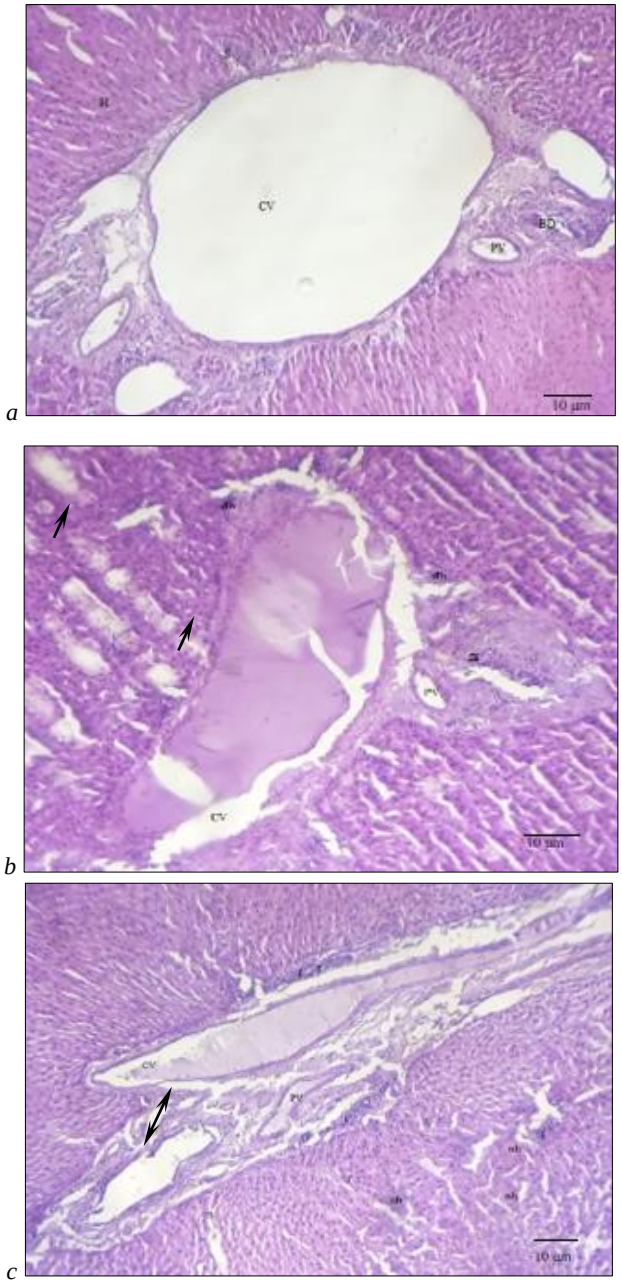


Fig. 1. Histological analysis of steatosis in liver sections stained with haematoxylin and eosin: *a* – control group; *b* – group with STZ-induced TYPE 2 DIABETES; *c* – group with STZ-induced T2D+Intracerebral haemorrhage; CV – central vein; PV – portal vein; BD – bile duct; S – sinusoid; H – hepatocytes; I – inflammatory; f – fibrous; nh – necrotic hepatocytes; dh – degenerative hepatocytes; microvesicular steatosis (arrowhead); portal tract convergence with deformation (double-headed arrow); scale bar = 10 µm

In rats with STZ-induced type 2 diabetes complicated by intracerebral haemorrhage treated with metformin (Fig. 2b), moderate microvesicular steatosis persisted, but the number of vacuoles was markedly reduced compared to the STZ+intracerebral haemorrhage group (Fig. 1c). Necrosis was absent, and intracerebral haemorrhage was minimal. The trabecular structure was partially restored, and hepatocyte nuclei appeared more uniform.

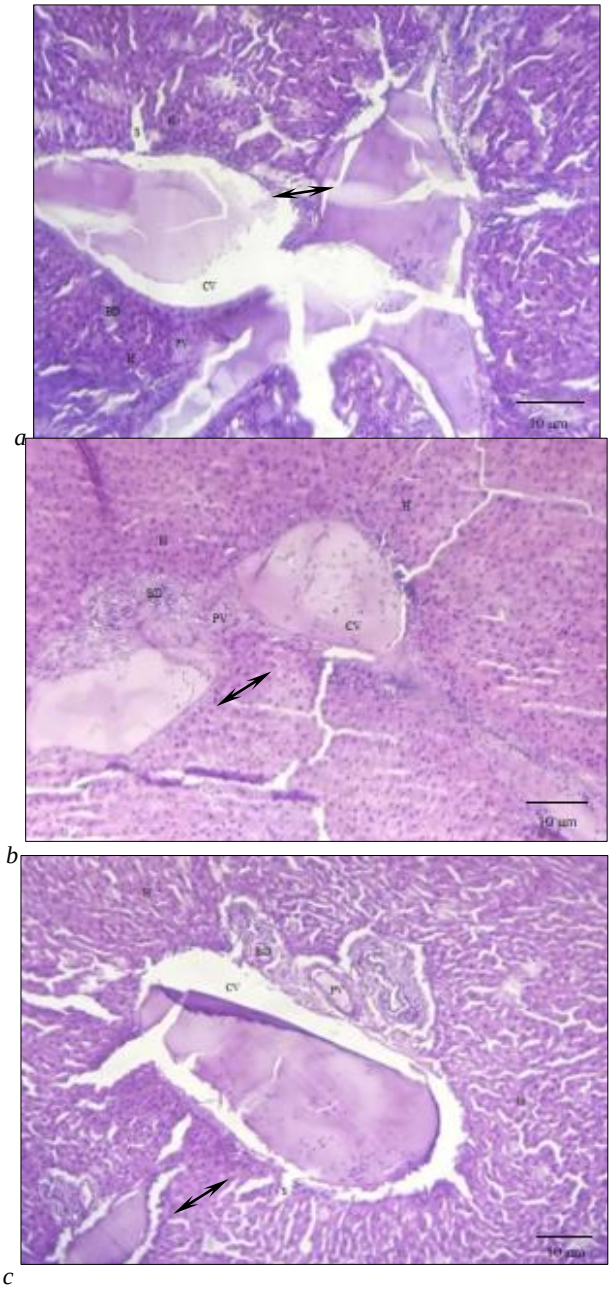


Fig. 2. Histological analysis of liver sections stained with haematoxylin and eosin: *a* – group with STZ-induced T2D+intracerebral haemorrhage+Rosuvastatin; *b* – group with STZ-induced T2D+intracerebral haemorrhage+Metformin; *c* – group with STZ-induced T2D+intracerebral haemorrhage+1,25$(OH)_2D_3$; CV – central vein; PV – portal vein; BD – bile duct; S – sinusoid; H – hepatocytes; portal tract convergence with deformation (double-headed arrow); scale bar = 10 µm

In rats with STZ-induced type 2 diabetes complicated by intracerebral haemorrhage treated with 1,25$(OH)_2D_3$ (Fig. 2c), lobular architecture was nearly restored. Hepatocytes exhibited well-defined boundaries and homogeneous cytoplasm, with only occasional cells containing small vacuoles (mild microvesicular steatosis). No signs of fibrosis, necrosis, or inflammation were detected. Vessels were not dilated.

The results of changes in biochemical parameters of lipid metabolism (total cholesterol and triglyceride concentrations) in the liver of rats in the STZ-induced type 2 diabetes model and under treatment conditions are shown inTable 1. STZ-induced diabetes led to increases in hepatic total cholesterol (~14%) and triglycerides (~130%). Intracerebral haemorrhage in the context of diabetes further exacerbated dyslipidaemia, with elevations in total cholesterol (~36%) and triglycerides (~146%). Rosuvastatin decreased total cholesterol levels

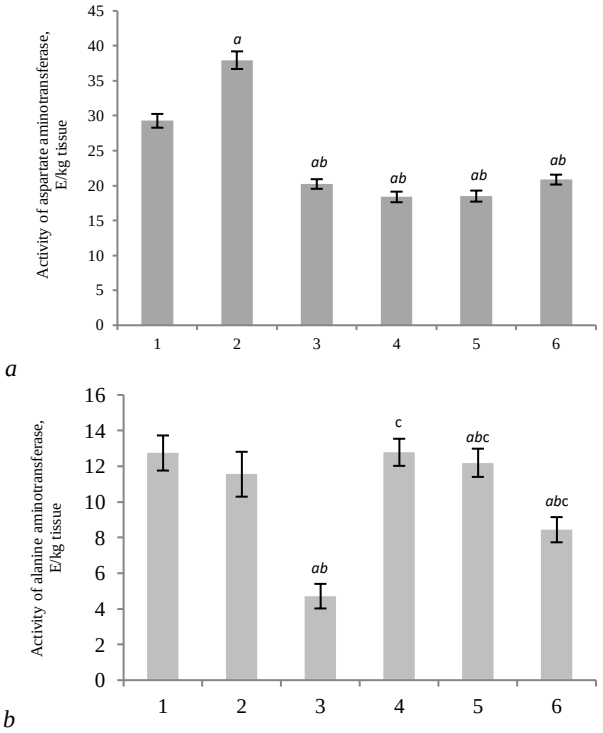
(compared to the control, ~40%) and triglyceride concentrations to within the control range. Metformin reduced total cholesterol (by ~27% compared to the control) and triglycerides (to levels above ~50% of the control) but did not fully restore them to normal levels.

Table 1
Concentration of total cholesterol (TC) and triglycerides (TG) in rat liver (mmol/kg tissue) in the STZ-induced type 2 diabetes model and under treatment conditions (mean ± SD, n = 6)

Animals	TC, mmol/kg tissue	TG, mmol/kg tissue
Control	3.40 ± 0.10^{d}	20.03 ± 2.13^{a}
STZ-induced T2D	3.87 ± 0.06^{e}	46.20 ± 1.80^{c}
STZ-induced T2D+ICH	4.62 ± 0.08^{f}	49.32 ± 1.88^{c}
STZ-induced T2D+ICH+Rosuvastatin	2.05 ± 0.01^{a}	20.64 ± 1.78^{a}
STZ-induced T2D+ICH+Metformin	2.47 ± 0.02^{b}	30.06 ± 2.58^{b}
STZ-induced T2D+ICH+1,25(OH)$_2$D$_3$	2.88 ± 0.03^{c}	31.50 ± 3.25^{b}

Note: different letters in the table within each line indicate significant ($P < 0.05$) differences between groups according to Tukey's test results.

In the liver cytosolic fraction of STZ-induced type 2 diabetes rats, aspartate aminotransferase (AST) activity increased by 30%. Alanine aminotransferase (ALT) activity showed no significant change compared to controls (Fig. 3a, 3b Group 2).



a

b

Fig. 3. Activity of aspartate aminotransferase (*a*) and alanine aminotransferase (*b*) in the cytosolic fraction of rat liver across experimental groups (E/kg tissue): *1* – intact animals; *2* – animals with STZ-induced T2D; *3* – animals with STZ-induced T2D+intracerebral haemorrhage; *4* – animals with STZ-induced T2D+intracerebral haemorrhage+Rosuvastatin; *5* – animals with STZ-induced T2D+intracerebral haemorrhage+Metformin; *6* – animals with STZ-induced T2D+intracerebral haemorrhage+1,25(OH)$_2$D$_3$; different letters in the table within each line indicate significant ($P < 0.05$) differences between groups according to Tukey's test results; all values are presented as mean ± SD, n = 6

At the same time, in STZ-induced type 2 diabetes complicated by intracerebral haemorrhage, AST activity (Fig. 3a, Group 3) decreased by 31%, and alanine aminotransferase activity (Fig. 3b, Group 3) was markedly reduced by 63% compared to the control group. Correction of the pathological state with rosuvastatin (Fig. 3b, Group 4) and metformin (Fig. 3b, Group 5) in STZ-induced type 2 diabetes complicated by intracerebral haemorrhage revealed a similar trend: alanine aminotransferase activity in Rosuvastatin (Fig. 3b, Group 4) increased nearly 3-fold compared to the STZ+intracerebral haemorrhage group, with values approaching those of the control group. However, aspartate aminotransferase activity remained at the Group 3 level, approximately 2-fold lower than that of the control group. Aspartate aminotransferase activity showed a similar reduction trend to Groups 4 and 5 (Rosuvastatin and Metformin, respectively), in the group treated with 1,25(OH)$_2$D$_3$ (Fig. 3a, Group 6). However, alanine aminotransferase activity was restored less effectively than in Groups 4 and 5 (Fig. 3b).

Discussion

Experimental type 2 diabetes and intracerebral haemorrhage as a dual factor of disruption. Type 2 diabetes is a global medical and social problem, with a steadily increasing prevalence. Diabetes is one of the leading causes of mortality and disability, particularly due to damage to the cardiovascular system, kidneys, and liver. The present study established that streptozotocin-induced diabetes in rats was accompanied by significant disruption of hepatic lipid homeostasis, demonstrated by elevated triglyceride levels and a moderate increase in total cholesterol. These findings are consistent with the concept of hepatic insulin resistance, whereby reduced hepatocyte insulin sensitivity enhances lipolysis in adipose tissue and increases the influx of free fatty acids into the liver (Frias et al., 2019).

Disruption of spatial organisation and clustering of the insulin receptor within the membrane, as described by Dall'Agnese et al. (2022) and Lee et al. (2023), explains the mechanism underlying lipotoxicity and steatosis in insulin resistance and the morphological changes observed in our study.

Adding an intracerebral haemorrhage in the context of diabetes further aggravated these disturbances, elevating hepatic triglyceride and cholesterol content and leading to macrovesicular steatosis, necrosis, and haemorrhage. These findings align with well-documented changes in hepatic ischemia/hypoperfusion, in which hypoxia, impaired microcirculation, and oxidative stress amplify inflammatory and destructive processes in hepatocytes (Neri et al., 2020).

Morphological changes and hepatic lipid profile. In the control group (Fig. 1a), liver tissue exhibited typical lobular architecture. Hepatocytes retained their regular polygonal shape, distinct cell boundaries, evenly stained cytoplasm, and centrally located nuclei. Central veins and sinusoids were moderately dilated without signs of blood stasis. No evidence of dystrophic or necrotic processes, inflammatory infiltrates, steatosis, or fibrosis was observed. The lobular architecture remained intact.

In animals with streptozotocin (STZ)-induced type 2 diabetes (Fig. 1b), microvesicular steatosis developed, characterised by small lipid vacuoles in hepatocyte cytoplasm, indicating early disturbances in lipid metabolism. Focal areas of moderate lymphocytic infiltration, mild sinusoidal dilatation, and dystrophic changes in hepatocytes were noted. The trabecular structure was partially disrupted, although overall lobular architecture was largely preserved. Fibrosis was minimal or absent. These findings are consistent with previous reports (Ning et al., 2015; Marino et al., 2023).

In the group STZ+intracerebral haemorrhage (Fig. 1c), histological analysis revealed pronounced parenchymal injury. Hepatocytes have large lipid vacuoles that displace the nucleus at the cell periphery, indicative of macrovesicular steatosis. Numerous haemorrhagic foci with extravasated erythrocytes, inflammatory infiltrates, and necrotic changes in hepatocytes were observed. Periportal fibrous septa in the lobular stroma indicated activation of connective tissue processes. Lobular architecture was severely disrupted, characterised by deformed central veins and disorganised hepatocyte arrangement. Current publications frequently describe steatosis in STZ-induced diabetes and combined with high-fat diet models (Ning et al., 2015; Silvares et al., 2016; Sporea et al., 2020), as well as isolated reports of hepatic haemorrhage/ischemic injury in specific models (Marino et al., 2023). However, direct studies describing the synergistic effect of

STZ+intracerebral haemorrhage – transforming microvesicular into macrovesicular steatosis and inducing marked fibrosis and necrosis are absent in available databases.

The comparative analysis demonstrated that liver structure remained unchanged in control animals, whereas STZ-induced diabetes resulted in early microvesicular steatosis and a moderate inflammatory response. The addition of the haemorrhagic factor markedly aggravated the injury, causing macrovesicular fatty degeneration, necrotic and fibrotic changes. Thus, the combination of diabetic and haemorrhagic pathology results in synergistic hepatic parenchymal damage, with features of combined toxic ischemic injury.

Rosuvastatin effects in the STZ-induced model. In this model (Fig. 2a), rosuvastatin exhibited a hepatoprotective effect, reducing steatosis and preventing the development of necrosis, fibrosis and haemorrhage. The absence of pronounced pathological changes may indicate the effectiveness of therapy for combined injury (STZ+intracerebral haemorrhage). In rats with STZ-induced type 2 diabetes complicated by intracerebral haemorrhage, rosuvastatin (15 mg/kg) exerted a marked hepatoprotective and hypolipidemic effect.

Histological examination of the liver revealed attenuation of steatosis, preservation of hepatocyte structural organisation, and reduced inflammatory response under rosuvastatin therapy. These findings are consistent with previous experimental data on the pleiotropic effects of statins (Heeba & Hamza, 2015; Fahmy et al., 2022).

Biochemical analysis (Table 1) confirmed the morphological observations: rosuvastatin demonstrated the most pronounced hypolipidemic effect among the therapeutic approaches studied, normalising hepatic levels of total cholesterol and triglycerides. A reduction in cholesterol concentration to approximately 40% below control may reflect not only inhibition of HMG-CoA reductase but also secondary attenuation of lipid infiltration into hepatocytes, thereby improving intracellular lipid homeostasis. Similar effects were described by-Fahmy et al. (2022), who reported that rosuvastatin reduces hepatic lipotoxicity by suppressing inflammatory and oxidative stress.

Metformin effects in the STZ-induced model. Metformin (Fig. 2b) demonstrated a moderate reduction in hepatic lipid burden, normalisation of total cholesterol, but partial persistence of hypertriglyceridemia (Table 1). This corresponds to activation of AMP-activated protein kinase (AMPK), which suppresses gluconeogenesis, reduces lipogenesis, and improves insulin resistance (Goel et al., 2022; Dutta et al., 2023). Metformin also exhibits cardioprotective and renoprotective properties (El Messaoudi et al., 2011; Dziubak et al., 2018), which enhance its relevance in comprehensive therapy.

Vitamin D effects in the STZ-induced model. In our experiment, Vitamin D demonstrated a moderate hepatoprotective effect. Significant improvement in the morphological picture was observed (Fig. 2c): moderate microvesicular steatosis; reduced, though not eliminated, inflammatory infiltration; absence of necrosis; and partial preservation of lobular architecture, consistent with findings reported by Kim (2024).

Partial normalisation of the lipid profile (Table 1): total cholesterol and triglyceride levels were reduced but did not reach the control levels. This correlates with studies demonstrating that Vitamin D modulates inflammation (Ravaioli et al., 2022; Shymanskyi et al., 2025), insulin sensitivity, oxidative stress (Kim et al., 2025), and the regulation of genes involved in lipid metabolism via Vitamin D Receptor-mediated pathways (Ning et al., 2015). Under hyperglycaemic and non-alcoholic fatty liver disease conditions, these mechanisms contribute to attenuation of steatosis (Farrash et al., 2024). Thus, Vitamin D exerted a positive but moderate hepatoprotective effect. It reduced the severity of fatty degeneration and inflammation; however, its efficacy was inferior to that of metformin and rosuvastatin, indicating a predominantly modulatory rather than a direct hypolipidemic action.

Comparative hepatoprotective effects of therapeutic agents. Application of corrective agents demonstrated varying degrees of hepatoprotective activity. Rosuvastatin proved to be the most effective, both in restoring the lipid profile (Table 1) and in improving hepatic histological structure (Fig. 2a); a similar effect was previously reported in a diet-induced steatosis model (Rondi et al., 2014). Metformin reduced cholesterol and steatosis (Table 1, Fig. 2b), which aligns with the mechanism of its AMPK activation (Goel et al., 2022; Dutta et al., 2023). But triglyceride normalisation was incomplete. Vitamin D, through Vitamin D Receptor-mediated pathways (Ning et al., 2015), reduced steatosis under hyperglycaemia/ non-alcoholic fatty liver disease conditions (Fig. 2c) and induced moderate normalisation of the lipid profile (Table 1). Thus, the results support current perspectives on insulin resistance and systemic stress in the development of diabetic fatty liver disease. Rosuvastatin has the strongest hepatoprotective potential in type 2 diabetes; metformin and Vitamin D may be considered auxiliary modulators of the metabolic response, particularly in the setting of diabetes combined with hypoxic-ischemic injury.

Functional state of the liver under experimental conditions. Aspartate aminotransferase and alanine aminotransferase activity measurements are commonly used to diagnose diseases of the liver, kidneys, and heart. Alanine aminotransferase activity predominates in the liver, whereas aspartate aminotransferase is more abundant in the myocardium. However, in the absence of other signs of cardiac injury in the blood, aspartate aminotransferase activity reflects significant pathological processes in hepatocytes, primarily involving organelle damage, such as mitochondrial damage.

It was established that in STZ-induced type 2 diabetes, liver cytosolic aspartate aminotransferase activity increased by 30%. Liver cytosolic alanine aminotransferase activity showed a non-significant decrease compared to the control group (Fig. 3b, Group 2), in STZ-induced type 2 diabetes complicated by intracerebral haemorrhage, aspartate aminotransferase activity (Fig. 3a, Group 3) decreased by 31% and alanine aminotransferase activity (Fig. 3b, Group 3) by 63% compared to the control, which may reflect profound impairment of hepatic metabolic and synthetic function rather than a classical cytolytic syndrome. Reduced transaminase activity has been observed in severe systemic injuries, such as ischemia and intracerebral haemorrhage (Pfisterer et al., 2024).

Correction of the pathological state with rosuvastatin (Fig. 3b, Group 4) and metformin (Fig. 3b, Group 5) in STZ-induced type 2 diabetes complicated by intracerebral haemorrhage revealed a similar trend: alanine aminotransferase activity increased nearly 3-fold, while in animals treated with Vitamin D (Fig. 3b, Group 6), alanine aminotransferase activity doubled compared to the STZ+intracerebral haemorrhage group. This dynamic may be interpreted as restoration of hepatocyte functional activity rather than hepatotoxicity. Rosuvastatin and metformin attenuated diabetes-induced tissue injury by suppressing oxidative stress, apoptosis, and inflammation, as reported by Heeba & Hamza (2015), the scientific group Kim et al. (2025), and Papanagnou et al. (2016).

Nevertheless, aspartate aminotransferase activity in the pharmacotherapy groups remained in Group 3 (Fig. 3a). At the same time, it was almost 2-fold lower than in the control. Stable, low aspartate aminotransferase levels might suggest incomplete recovery of liver mitochondrial function, as expected in severe combined injury with short treatment. These findings confirmed the rationale for using rosuvastatin by correcting comprehensive metabolic and morphofunctional liver disturbances in the setting of combined diabetes and intracerebral haemorrhage. Veluthakal et al. (2024) highlighted the role of mitochondrial dysfunction in the development of metabolic syndrome and progression to type 2 diabetes. However, clarifying the impact of type 2 diabetes and intracerebral haemorrhage on mitochondrial aspartate aminotransferase activity requires further experiments.

The results are consistent with experimental studies demonstrating cardio-, nephro-, and neuroprotective effects of statins in STZ-induced diabetes models (Rondi et al., 2014), as well as with data on the pleiotropic effects of statins independent of their lipid-lowering action (Zhang et al., 2018). At the same time, clinical studies indicate limited efficacy of statins in patients with terminal stages of liver disease (Pfisterer et al., 2024), underscoring the importance of disease context and stage.

Thus, the results of this study demonstrate that rosuvastatin in a model of STZ-induced type 2 diabetes complicated by intracerebral haemorrhage exerts a pronounced hepatoprotective effect, manifested by normalisation of lipid metabolism, reduction of morphological signs of damage, and partial restoration of hepatic functional activity.

These findings expand current understanding of the role of statins in the comprehensive correction of combined metabolic and vascular injury and justify further mechanistic investigations. In addition, the results confirm the potential efficacy of metformin and vitamin D as components of combined therapy for type 2 diabetes, including in the presence of concomitant hepatic pathology, and highlight their gradual restorative influence on liver function.

Structural determinants of the biological action of rosuvastatin, metformin, and vitamin D. The results obtained demonstrate that the hepatoprotective efficacy of rosuvastatin, metformin, and vitamin D in the STZ-induced type 2 diabetes model complicated by intracerebral haemorrhage is largely determined by their structural characteristics, which underline specific molecular mechanisms of action.

Rosuvastatin is a hydrophilic statin containing a polar sulphonamide group and a fluorinated phenyl ring, which confer high hepatic selectivity. The presence of the sulphonamide group facilitates polar active uptake by hepatocytes via organic anion transporting polypeptide (SLCO1B1) (Li et al., 2019; Domshyna & Kyrychenko, 2020), ensuring high intracellular drug concentrations. Inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase directly suppresses endogenous cholesterol synthesis (Grievink et al., 2019; Sporea et al., 2020; Christiansen et al., 2021). This structural feature explains the pronounced reduction in intrahepatic cholesterol and triglyceride levels observed in our study. Beyond its direct hypolipidemic action, the amphiphilic structure of rosuvastatin contributes to pleiotropic effects, including suppression of nuclear factor-κB-dependent inflammation, reduction of oxidative stress, and stabilisation of hepatocyte membranes (Rondi et al., 2014; Heeba & Hamza, 2015). These mechanisms are consistent with the morphological findings of our study, which demonstrated complete regression of steatosis, absence of necrosis, and restoration of lobular hepatic architecture.

Metformin is a small biguanide molecule with high hydrophilicity that does not directly interact with membrane receptors but readily enters hepatocytes via organic cation transporters (OCT1) (Corsini & Bortolini, 2013; Dyomshyna & Kyrychenko, 2020). Its structural simplicity underlies an indirect metabolic action mediated through the activation of AMP-activated protein kinase, the central regulator of cellular energy homeostasis (Goel et al., 2022).

The results showed normalisation of cholesterol levels and partial reduction of triglycerides, accompanied by attenuation of microvesicular steatosis but not complete regression. Such an effect is consistent with existing data indicating that metformin effectively suppresses lipogenesis but is not a direct inhibitor of lipid synthesis, unlike statins (Dutta et al., 2023).

Vitamin D is a steroid molecule like other steroids. Enabling interaction with the nuclear receptor and directing gene transcription. This structural feature determines the slower but multifaceted biological effects (Du et al., 2023; Reda et al., 2023). In our model, vitamin D induced moderate reductions in triglycerides and cholesterol, accompanied by a decrease in steatosis and inflammatory infiltration. Mechanistically, these effects are associated with suppression of the sterol regulatory element binding protein-1c-dependent lipogenesis, activation of peroxisome proliferator-activated receptor-α, and inhibition of the nuclear factor-κB-mediated inflammation, as previously demonstrated in experimental models of diabetes and non-alcoholic fatty liver disease (Ning et al., 2015; Reda et al., 2023).

The obtained results indicate that the effectiveness of pharmacological correction of hepatic disturbances in the STZ-induced type 2 diabetes model complicated by intracerebral haemorrhage is substantially determined by the chemical structure of the investigated compounds and their corresponding molecular targets.

Rosuvastatin, through structurally direct inhibition of cholesterol synthesis and pronounced pleiotropic effects, provides the most complete normalisation of lipid metabolism and morphological restoration of the liver (Burtis et al., 2012; Fahmy et al., 2022). Metformin, a small hydrophilic molecule and energy regulator, exerts a moderate hepatoprotective effect via AMP-activated protein kinase-dependent mechanisms (Frias et al., 2019). Vitamin D, as a steroid-like secosteroid hormone, produces slower but stable anti-inflammatory and antisteatosis effects through transcriptional regulation (Du et al., 2023; Reda et al., 2023). Thus, the established link between chemical structure and mechanisms of action substantiates a differentiated approach to pharmacological correction of hepatic disturbances in conditions of diabetes complicated by vascular injury and confirms the potential of combined therapeutic strategies.

Clinical relevance. The results of this experimental study highlight the liver as a critical target organ in type 2 diabetes, particularly under additional systemic stress such as intracerebral haemorrhage. The demonstrated aggravation of hepatic lipid dysregulation and structural damage in this combined pathological context supports the need for careful monitoring of liver function and lipid metabolism in patients with diabetes experiencing acute vascular or intracerebral haemorrhage complications. The pronounced hepatoprotective effect of rosuvastatin, compared with metformin and vitamin D, underscores the potential clinical importance of lipid-lowering therapy by pleiotropic properties in diabetic patients at high risk of hepatic involvement. Although extrapolation from animal models to clinical practice should be made with caution, these findings provide a mechanistic rationale for considering differentiated or combination therapeutic strategies protecting the liver in complex metabolic-vascular conditions.

Conclusion

This study demonstrates that STZ-induced type 2 diabetes is accompanied by clinically relevant disturbances of hepatic lipid metabolism and structural integrity, which are substantially aggravated by haemorrhage. The combined metabolic and vascular burden leads to pronounced liver damage, highlighting the liver as a critical target organ in complex diabetic comorbidities.

Among the pharmacological interventions investigated, rosuvastatin exhibited the most pronounced hepatoprotective effect, effectively restoring hepatic lipid balance and preserving liver architecture in diabetes complicated by intracerebral haemorrhage. These findings emphasise the clinical relevance of statins beyond lipid-lowering, particularly in patients with type 2 diabetes and concomitant vascular complications. Metformin provided partial protection, improving individual metabolic parameters and attenuating liver damage. The effect of vitamin D was limited, indicating its supportive rather than primary therapeutic potential in this setting.

Overall, the results support the necessity of active pharmacological correction of hepatic dysfunction in type 2 diabetes complicated by intracerebral haemorrhage. The superior efficacy of rosuvastatin underscores its potential role as an integral component of comprehensive management strategies for patients with combined metabolic and vascular disorders. These findings provide experimental justification for further clinical evaluation of statin-based approaches aimed at preventing or attenuating liver involvement in complex diabetic pathology.

The authors declare that they have no competing interests.

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