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Hepatoprotective Effects of a Ruthenium(II) Schiff Base Complex in Rats with Diet-Induced Prediabetes

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ABSTRACT

Background: Progressive insulin resistance in a prediabetic state has been reported to be the predominant causative factor for the development of nonalcoholic fatty liver disease. The combination of dietary modification and pharmacotherapy has been recommended to manage diabetic liver complications. However, poor patient compliance and toxicity of current drug therapy on liver function still results; thus, newer alternative drugs are required.
Objective: This study sought to investigate the hepatoprotective effects of the ruthenium(II) Schiff base complex in the presence and absence of dietary intervention in a diet-induced pre-diabetic rat model.
Methods: Prediabetic rats were randomly allocated to respective treatment groups. The ruthenium-based compound (15 mg/kg) was administered to the prediabetic rats in both the presence and absence of dietary intervention once a day every third day for 12 weeks.
Results: The administration of the ruthenium compound in both the presence and absence of dietary intervention resulted in the restoration of liver and body weights. This treatment also reduced liver damage enzyme biomarkers, bilirubin, and sterol regulatory element binding protein 1c concentrations in the plasma.
Conclusions: The ruthenium(II) complex showed beneficial effects as it ameliorated and prevented the progression of diabetes-related liver derangements while eliminating the hepatotoxicity associated with the use of metal compounds. However, further studies are still required to further determine the physiological mechanisms behind this effect.

Introduction

The liver plays an important role in the maintenance of systemic lipid and glucose homeostasis.[1] However, liver damage leads to dysregulation of both lipid and glucose metabolism and is a serious complication among patients with prediabetes and diabetes.[2] Liver damage is associated with several abnormalities, such as abnormal glycogen deposition, nonalcoholic fatty liver disease (NAFLD) and elevated plasma concentrations of liver damage biomarkers.[3,4] In pre-diabetes, insulin resistance and compensatory hyperinsulinaemia have been shown to be the predominant causative factors of liver pathology.[5–7] The insulin resistance is said to impair the antilipolytic action of insulin in adipose tissue, which leads to increased release of free fatty acids.[8–11] The elevated plasma concentrations of insulin, glucose and fatty acids then impairs the β-oxidation of fatty acids and enhances de novo lipogenesis in the liver under the control of a specific transcription factor steroid regulatory elementary binding protein-1c (SREBP-1c).[4,12,13] The presence of abnormal plasma levels of liver enzymes, such as alanine transaminase (ALT) and aspartate transaminase (AST) levels resemble liver injury and are found to be increased in patients with prediabetes.[4,14] In addition, studies have shown that bilirubin, which has been shown to have protective effects against cardiovascular complications, is lowered in diabetes.[15,16] Furthermore, the histopathological changes in patients with diabetes include increased hepatic glycogen concentration, accumulation of hepatic fat, and increased liver size.[13,17,18] Dietary modifications and pharmacotherapy have been used to manage NAFLD as well as nonalcoholic steatohepatitis liver complications

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that aid in reducing body weight and improving insulin sensitivity in the liver and periphery.[7,19,20] However, the clinical value of metformin, pioglitazone, thiazolidinediones together with other treatments such as betaine, atorvastatin, losartan, and orlistate is very subjective. Patients taking these drugs should be closely monitored due to possible contraindications with diabetes mellitus medications and the vulnerable condition of the liver during the drug detoxification process.[20,21] Ruthenium (II)-derived complex has been shown to possess anti-inflammatory properties and exhibited protective effect against lipopolysaccharide-induced liver injury in mice through mediation the inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells signaling pathways.[22] Furthermore, recent studies have reported that ruthenium(II) complex with a diimine uracil chelating ligand possesses antidiabetes properties that improved glycemic control, insulin sensitivity, and decreased risk of developing diabetes-related cardiovascular diseases in diet-induced prediabetic rats.[23,24] However, the effects of this metal complex on hepatic complications in the prediabetic state remain unknown. Hence, this study sought to investigate the hepatoprotective effects of the ruthenium(II) Schiff base complex in the presence and absence of dietary intervention in a diet-induced prediabetic rat model.

Materials and Methods

Chemicals and drugs

All chemicals and drugs were of analytical grade and purchased from standard commercial suppliers. Previous studies showed that the dose of the ruthenium complex used in this study was nontoxic.[23,24] *Synthesis of ruthenium(II) Schiff base complex*

The synthesis of ruthenium(II) Schiff base complex, $[Ru^{II}(H_3ucp)Cl(PPh_3)]$ ($H_4ucp = 2,6$-*bis*-((6-amino-1,3-dimethyluracilimino) methylene) pyridine), was done in our laboratory as previously reported.[25]

Animals and housing

In this study, 36 male Sprague-Dawley rats (150–180 g) were used. The animals were housed in a room with a 12-hour light/12-hour dark cycle, at room temperature (25°C), for the duration of the study. The animals in each group had access to food and water ad libitum. All animal procedures and housing conditions were approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (ethical clearance No.: AREC/038/016M).

Induction of prediabetes

The animals were randomly assigned to the following diet groups: normal diet with drinking water and high-fat high-carbohydrate diet with drinking water supplemented with 15% fructose (AVI Products Ltd, Waterfall, South Africa). Prediabetes was induced by allowing the animals to feed on the high-fat high-carbohydrate diet for 20 weeks as previously described.[26] Glucose tolerance was evaluated 5 days after the 20 weeks of induction with a well known, established laboratory protocol, the oral glucose tolerance test to determine prediabetes according to the American Diabetes Association criteria.[23] The rats with fasting blood glucose >5.6 mmol/L were considered prediabetic and grouped further for pharmacologic studies. The animals that were fed the normal diet were also tested and were found to be normoglycemic and without prediabetes. The treatment started on the subsequent day and this was considered as the first day of treatment.

Experimental design

The study consisted of 2 main groups, the nonprediabetic animals (n = 6) and the prediabetic animals (n = 30). After 20 weeks of induction, the prediabetic animals were divided into the following 5 groups (n = 6): the first group was fed a high-fat high-carbohydrate diet without treatment. The second group was fed on a high-fat high-carbohydrate diet and treated with oral dose of metformin (500 mg/kg) (Sigma-Aldrich, St Louis, Missouri). The third group was fed a normal diet and treated with oral dose of metformin (500 mg/kg) (Sigma-Aldrich). The fourth group was fed a high-fat high-carbohydrate diet and treated with subcutaneous injection of ruthenium(II) complex (15 mg/kg), whereas the fifth group was fed a normal diet and treated with subcutaneous injection of ruthenium(II) complex (15 mg/kg). The animals were treated once a day every third day at 9:00 AM for 12 weeks. Throughout the 12-week treatment period, parameters such as body weight and fasting blood glucose were monitored every 4 weeks.

Blood collection and tissue harvesting

All animals were anesthetised with Isofor (100 mg/kg) (Safeline Pharmaceuticals Ltd, Roodeport, South Africa) using a gas anesthetic chamber (Biomedical Resource Unit, University of KwaZulu-Natal, Durban, South Africa) and allowed to inhale for 3 minutes. Blood was collected by cardiac puncture and then injected into individual precooled heparinized containers. The blood was then centrifuged for plasma collection (Eppendorf centrifuge model 5403; Eppendorf North America, Hauppauge, New York) at 4°C, 503 g for 15 minutes. Thereafter, liver was removed, weighed and halved into 2 liver tissue samples. One liver tissue sample was snap frozen in liquid nitrogen before storage in a BioUltra freezer (Snijers Scientific, Tilburg, the Netherlands) at –80°C until biochemical analysis, and the other liver tissue sample was kept in 10% formalin buffer for histopathological examination.

Biochemical analysis

Plasma AST, ALT, and total bilirubin concentrations were measured using the Catalyst One Chemistry Analyzer (IDEXX Laboratories, Westbrook, Maine). The concentration of SREBP-1c was analysed using specific ELISA kits in accordance with the manufacturer's instructions (Elabscience and Biotechnology, Wuhan, China). The kits included a micro-ELISA plate that was coated with antibodies specific to SREBP-1c. Standards and samples were pipetted into the appropriate wells of the micro-ELISA plate and incubated for 90 minutes at 37°C. This was followed by the addition of the relevant biotinylated detection antibody (100 µL). After 60 minutes of incubation at 37°C, avidin–horseradish peroxidase conjugate (100 µL) was added to each microplate well. After a further 30 minutes of incubation at 37°C, the unbound components were washed away using the wash buffer provided. Substrate solution (100 µL) was added to each microplate well and, after 15 minutes of incubation at 37°C, the stop solution (50 µL) was added. Optical density was measured using a nano spectrophotometer (BMG Labtech, Ortenburg, Germany) at 450 nm. The concentrations of SREBP-1c in the samples were extrapolated from a standard curve.

Glycogen assay

Glycogen analysis was performed in liver tissues. Glycogen assay was conducted using a well-established laboratory protocol.[23] Liver tissues (50 mg) were weighed and heated with potassium hydroxide (30%, 2 mL) at 100°C for 30 minutes. Thereafter, sodium

Table 1
Effects of the ruthenium complex on liver glycogen concentration of prediabetic animals for a treatment period of 12 wk (n = 6 in each group).

Group	Hepatic glycogen (nmol/g protein)
	mean (SEM)
NPD	1.88 (0.40)
PD	2.25 (0.51)*
MTF + HFHC	2.38 (1.12)*
MTF + ND	1.41 (0.12)†
RU + HFHC	2.18 (0.72)*
RU + ND	1.62 (0.23)†

MTF + HFHC = metformin and high-fat high-carbohydrate; MTF + ND = metformin and normal diet; NPD = nonprediabetic; PD = prediabetes; RU + HFHC = ruthenium(II) complex and high-fat high-carbohydrate; RU + ND = ruthenium complex and normal diet.
* $P < 0.05$ compared with NPD.
† $P < 0.05$ compared with PD.

sulfate (10%, 0.194 mL) was added to stop the reaction and allowed to cool. For glycogen precipitation, the cooled mixture (200 µL) was aspirated and mixed with ethanol (95%, 200 µL). The precipitated glycogen was pelleted, washed and redissolved in water (1 mL). Thereafter, anthrone (0.5 g dissolved in 250 mL sulphuric acid, 4 mL of anthrone) was added and boiled for 10 minutes. After cooling the absorbance was read using the Spectrostar Nano spectrophotometer (BMG Labtech, Baden-Württernberg, Germany) at 620 nm. The glycogen concentrations were calculated from a glycogen standard curve.

Hepatic histology examination

Liver histology was analyzed using a previously described protocol.[27] Liver tissues were immediately fixed in 10% formalin buffer after removal from the animals. The tissue samples were embedded in paraffin wax after alcohol dehydration process. By using microtome, 5 µm sections of liver tissue were taken and were stained by haematoxylin and eosin stain. The processed tissue sections were then visualized and captured using a Leica Scanner, SCN400 and Slide Path Gateway LAN software for analysis (Leica Microsystems CMS, Wetzlar, Germany).

Statistical analysis

Data were reported as mean (SEM). GraphPad Prism Software (version 5) (San Diego, California) was used to conduct statistical analysis. The differences between control and treated groups were analysed using One-way ANOVA followed by Tukey-Kramer. Values of $P < 0.05$ show statistical significance between the compared groups.

Results

Liver glycogen

In comparison with the nonprediabetic group, the prediabetic animals group showed a significant increase in liver glycogen concentration ($P < 0.05$) (Table 1). The administration of ruthenium(II) complex plus high-fat high-carbohydrate diet resulted in a significant increase in liver glycogen concentration by comparison to the nonprediabetic animals group ($P < 0.05$) (Table 1). The administration of ruthenium(II) complex and normal diet significantly ($P < .05$) reduced liver glycogen when compared with the prediabetic animals group. A similar effect was observed with the metformin plus normal diet treated group when compared with the prediabetic animals group ($P < 0.05$) (Table 1).

Table 2
The influences of the ruthenium complex on liver and body weight of prediabetic animals for a treatment period of 12 wk (n = 6 in each group).

Group	Initial body weight (g)	Final body weight (g)	Liver weight (g)
	mean (SEM)		
NPD	173 (1.45)	384 (6.30)	11.53 (0.14)
PD	176 (1.73)	680 (8.08)*	24.33 (1.23)*
MTF + HFHC	172 (1.22)	501 (5.06)*,†	17.34 (0.86)*,†
MTF + ND	177 (0.98)	443 (3.90)†	13.35 (1.37)†
RU + HFHC	172 (1.45)	490 (5.09)*†	16.23 (0.65)*,†
RU + ND	175 (1.67)	435 (2.61)†	13.20 (0.91)†

MTF + HFHC = metformin and high-fat high-carbohydrate; MTF + ND = metformin and normal diet; NPD = nonprediabetic; PD = prediabetes; RU + HFHC = ruthenium(II) complex and high-fat high-carbohydrate; RU + ND = ruthenium complex and normal diet.
* $P < 0.05$ compared with NPD.
† $P < 0.05$ compared with PD.

Liver and body weight

By comparison with the nonprediabetic animals group, the prediabetic animals group showed a significant increase in both liver and body weight ($P < 0.05$) (Table 2). The administration of ruthenium(II) complex plus a high-fat high-carbohydrate diet resulted in a significant increase in both liver and body weight when compared with the nonprediabetic animals group ($P < 0.05$) (Table 2). However, when compared with the prediabetic animals group, administration of ruthenium complex in the presence and absence of dietary intervention showed a significant decrease in body weight with further decrease in liver weight in the ruthenium-treated prediabetic animals ($P < 0.05$) (Table 2). In addition, the metformin-treated animals displayed similar results when compared with the nonprediabetic animals group and the prediabetic animals groups ($P < 0.05$) (Table 2).

Plasma liver function enzymes AST and ALT concentration

The untreated prediabetic animals group showed a significant increase in plasma AST concentration when compared with the nonprediabetic animals group over the treatment period ($P < .05$) (Figure 1A). However, when compared with the prediabetic animals group, both the groups treated with the metal-based drug resulted in a significant decrease in AST concentration ($P < .05$) (Figure 1A) . The metformin-treated groups showed a restored AST concentration when compared with the prediabetic animals group ($P < .05$) (Figure 1A). In addition, there were no significant changes in ALT concentration in the prediabetic animals group when compared with the nonprediabetic animals group ($P < .05$) (Figure 1B). Administration of ruthenium complex in the presence and absence of dietary intervention showed a significant decrease in ALT concentration when compared with both the nonprediabetic animals and the prediabetic animals groups, with the ruthenium complex and normal diet group showing more effective results when compared with the nonprediabetic animals group ($P < .05$) (Figure 1B). However, the metformin-treated groups showed a significant increase in ALT concentration when compared with both the nonprediabetic animals group and the prediabetic animals groups ($P < .05$) (Figure 1B).

Plasma total bilirubin concentration

The prediabetic animals group showed a significantly decreased plasma total bilirubin concentration when compared with the nonprediabetic animals group over the treatment period ($p < .05$) (Figure 2). Both ruthenium(II) complex plus high-fat high-carbohydrate diet and ruthenium(II) complex plus normal diet

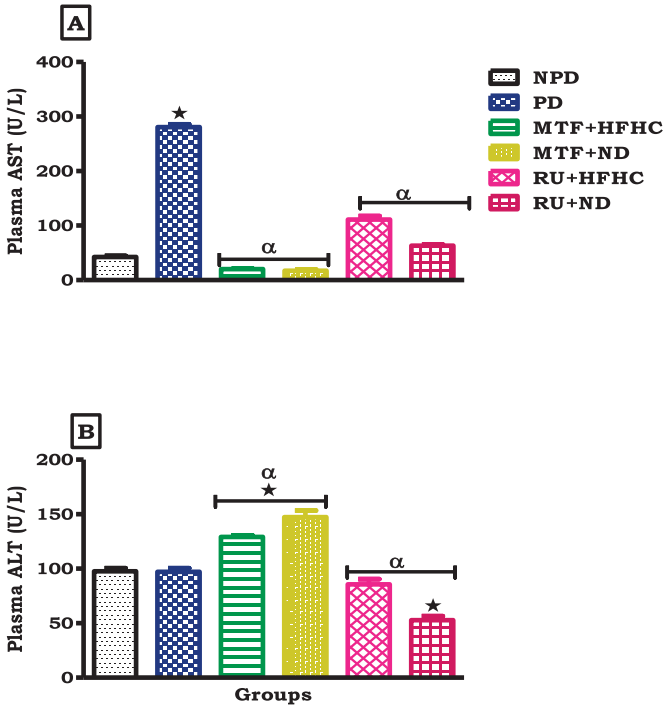

Figure 1. The effects of the ruthenium(II) complex. (A) Aspartate transaminase (AST) and (B) alanine transaminase (ALT) concentrations of prediabetic animals during the treatment period. Values are presented as mean (SEM) (n = 6 in each group). MTF + HFHC = metformin and high-fat high-carbohydrate; MTF + ND = metformin and normal diet; NPD = nonprediabetic; PD = prediabetes; RU + HFHC = ruthenium(II) complex and high-fat high-carbohydrate; RU + ND = ruthenium(II) complex and normal diet. $^{*}P < 0.05$ compared with NPD; $^{\alpha}P < 0.05$ compared with PD.

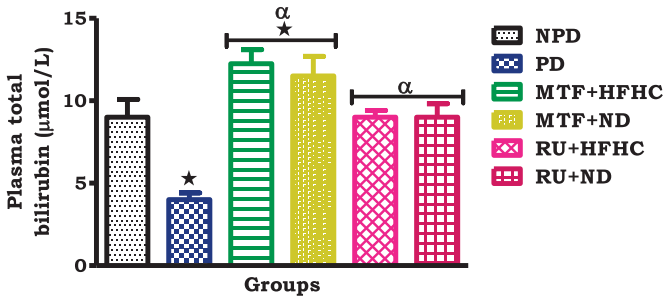

Figure 2. The effects of the ruthenium(II) complex on total bilirubin concentration of prediabetic animals during the treatment period. Values are presented as mean (SEM) (n = 6 in each group). MTF + HFHC = metformin and high-fat high-carbohydrate; MTF + ND = metformin and normal diet; NPD = nonprediabetic; PD = prediabetes; RU + HFHC = ruthenium(II) complex and high-fat high-carbohydrate; RU + ND = ruthenium(II) complex and normal diet. $^{*}P < 0.05$ compared with NPD; $^{\alpha}P < 0.05$ compared with PD.

resulted in a significant increased plasma total bilirubin concentrations when compared with the prediabetic animals group ($p < .05$) (Figure 2). The metformin-treated groups showed a significant increased plasma total bilirubin concentrations when compared with both the nonprediabetic and prediabetic groups ($p < .05$) (Figure 2).

Plasma SREBP-1c concentration

The prediabetic animals group showed significantly increased plasma SREBP-1c concentration when compared with the nonprediabetic animals group over the treatment period ($P < 0.05$) (Figure 3). The ruthenium complex plus high-fat high-carbohydrate diet group had significant high plasma SREBP-1c concentration

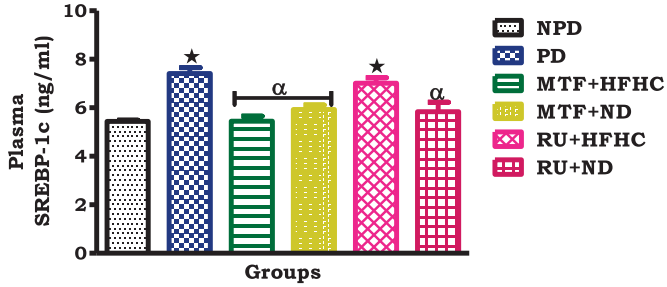

Figure 3. The effects of the ruthenium(II) complex on steroid regulatory elementary binding protein-1c (SREBP-1c) concentration of prediabetic animals during the treatment period. Values are presented as mean (SEM) (n = 6 in each group). MTF + HFHC = metformin and high-fat high-carbohydrate; MTF + ND = metformin and normal diet; NPD = nonprediabetic; PD = prediabetes; RU + HFHC = ruthenium(II) complex and high-fat high-carbohydrate; RU + ND = ruthenium(II) complex and normal diet. $^{*}P < 0.05$ compared with NPD; $^{\alpha}P < 0.05$ compared with PD.

when compared with the nonprediabetic animals group ($P < 0.05$) (Figure 3). When compared with the prediabetic animals group, the administration of ruthenium complex plus normal diet reduced plasma SREBP-1c concentration to within that of nonprediabetic animals group ($P < 0.05$) (Figure 3). The metformin-treated groups showed significant decrease in plasma SREBP-1c concentrations when compared with the prediabetic animals group ($P < 0.05$) (Figure 3).

Histology of the liver

Liver histology analysis revealed that there was increased lipid droplet accumulation, hepatocytes ballooning and lobular disarray in the prediabetic group animals (Figure 4B) compared with nonprediabetic animals (Figure 4A). The administration of ruthenium compound to the prediabetic animals (Figure 4E and F) reduced lipid accumulation and restored hepatocytes size and shape in the prediabetic metal complex-treated animals compared with prediabetic control animals (Figure 4B). Results showed that there was no remarkable difference between ruthenium complex plus high-fat high-carbohydrate diet and ruthenium complux plus normal diet treated animals in liver histology sections (Figure 4E–F). In addition, the metformin-treated animals showed similar results (Figure 4C and D).

Discussion

In the current study, we investigated the effects of a ruthenium(II) Schiff base complex on diabetes-related hepatic complications in the presence and absence of dietary intervention. The liver plays a key role in regulating glucose metabolism and becomes compromised during derangements, such as NAFLD and prediabetes.[28–30] The results obtained in this study showed a significant increase in hepatic glycogen content in the prediabetic animals group by comparison with the nonprediabetic animals control group. Studies show that the increased glycogen content in patients with obesity and prediabetes is associated with decreased peripheral insulin sensitivity.[31,32] The administration of the ruthenium(II) complex reduced hepatic glycogen concentration in the prediabetic animals. The restoration of hepatic glycogen concentrations could be attributed to the metal-based compound, ameliorating insulin sensitivity thus improving glycemic control.[23] Excessive accumulation of glycogen and fatty acids in the hepatocytes have been reported to lead to hepatomegaly with steatosis being the major causative factor in patients with diabetes.[33] This study has shown that the administration of this ruthenium(II) complex in both the presence and absence of dietary intervention ameliorated body weight gain in prediabetic rats. A reduction in body weight

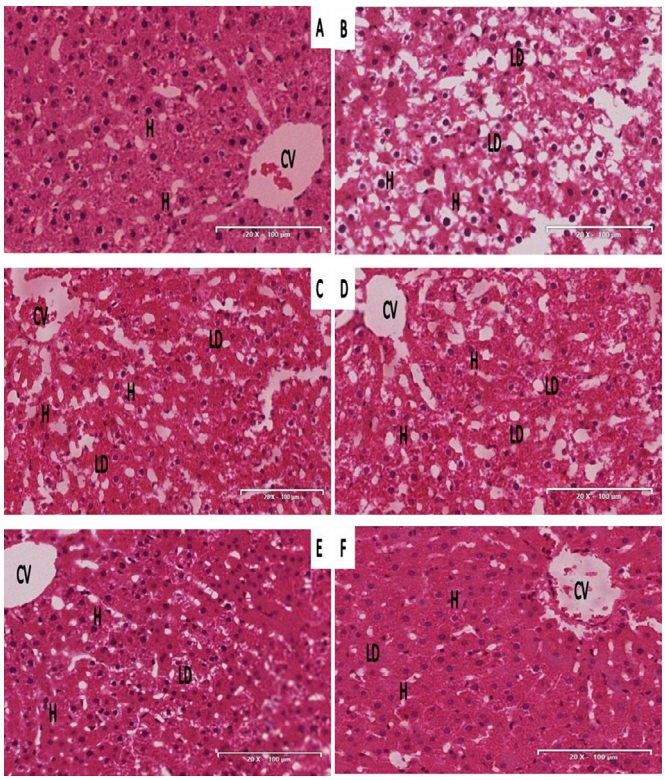

Figure 4. The effects of the ruthenium(II) complex on liver histopathological analyses in prediabetes (PD) treated animals during the treatment period (hematoxylin and eosin stain magnification 20 × (100 μm). (A) Nonprediabetic (NPD) group. (B) PD group. (C) Metformin and high-fat high-carbohydrate (MFT+HFHC) group. (D) Metformin and normal diet (MFT + ND) group. (E) Ruthenium(II) complex and high-fat high-carbohydrate (RU + HFHC) group. (F) Ruthenium(II) complex and normal diet (RU+ND) group. CV = central vein; H = hepatocyte; LD = lipid droplets.

of as low as 8% is associated with reduced steatosis and improved insulin tolerance.[30] The results of the present study further revealed that administration of the ruthenium(II) complex resulted in a significant decrease in the liver weights compared with the prediabetic animals group. Ruthenium(II) Schiff base complex has been shown to ameliorate caloric intake through the reduction of plasma ghrelin levels in diet-induced prediabetic rats.[23] Furthermore, several studies suggest that increased liver weights in prediabetes and NAFLD are associated with increased hepatic lipid accumulation.[8–11] Indeed, histological analysis showed that there was increased hepatic lipid droplet accumulation in the untreated prediabetic rats. However, the administration of the ruthenium(II) complex in both the presence and absence of dietary intervention resulted in a decrease in lipid accumulation. Taken together, these results could suggest that the ability of this compound to restore insulin sensitivity in the prediabetic state restores body weights while reducing hepatic accumulation of glycogen and fats. This would then result in a reduction in liver weights and the prevention of hepatomegaly that is associated with NAFLD.[34]

Plasma AST and ALT are enzyme biomarkers used to monitor the liver's structural integrity and aids in the clinical diagnosis of NAFLD and other liver toxicity conditions.[35] Generally, high-calorie diets increase plasma concentration of these enzymes through the induction of oxidative stress in the liver.[35] Indeed, the results of the present study showed that the prediabetic animals group had significantly increased plasma levels of AST compared with the nonprediabetic control group. These results suggest that diet-induced prediabetes can has deleterious effects on the liver due to production of free radicals and reactive oxygen species that further trigger the inflammatory response mechanism.[36] On the other hand, there was no significant change in plasma ALT levels in the prediabetic animals group when compared with the nonprediabetic group. However, due to the age of the rats, which were 36 weeks old at the end of the experiment, we speculate that the observed result on ALT level may be due to age. Literature evidence has shown that oxidative stress increases with age which then trigger the inflammatory response mechanism and reduces cellular antioxidant capacity, thus leading to mutation and DNA damage that can be a predisposing factor to impair liver function.[37,38] Furthermore, the normal diet has a considerable amount of carbohydrates. Ingested carbohydrates are a major stimulus for hepatic de novo lipogenesis and more likely to directly contribute to NAFLD and impair liver function enzymes than dietary fat intake.[39] However, we acknowledge that further mechanisms are needed to elucidate the increase in ALT levels in the nonprediabetic rats. The administration of metal complexes is often associated with increased plasma AST and ALT levels, suggesting liver toxicity.[40] Pharmacotherapy for diabetes-related liver disorders is often combined with dietary intervention that involves the consumption of low-calorie diets.[30] However, there is reported low patient compliance in terms of dietary intervention because patients tend to heavily rely on the pharmacologic treatments and thus reduce the efficacy of the drugs.[41]

However, the administration of the ruthenium(II) complex, in both the absence and presence of dietary intervention led to decreased plasma AST and ALT levels in the prediabetic treated rats group. In addition, treatment with the metal compound with dietary intervention displayed more effective results in terms of lowering ALT levels in prediabetic rats. A recent study by Hsia et al[22] demonstrated the anti-inflammatory properties of a novel ruthenium compound, via inhibiting inflammatory mediators (nitric oxide and nitric oxide synthase) and proinflammatory cytokines (tumor necrosis factor-α and interleukin-1β)in RAW 264.7 cells. Additionally, the metal compound also displayed protective effect against lipopolysaccharide-induced liver injury in mice, thus protecting progressive liver damage.[22] Moreover, the findings of the study could suggest that the integration of the Schiff base ligand within the coordination sphere of this ruthenium(II) complex could possibly protect the liver from toxic and proinflammatory effects because metal compounds complexed with organic ligands have been found to be less toxic.[25] Notably, the metformin-treated prediabetic animals showed decrease in AST levels in both the presence and absence of dietary intervention. In contrast, there was an increase in plasma ALT levels in metformin-treated animals compared with both nonprediabetic and prediabetic control groups. Metformin has been reported to improve liver injury but could not prevent fibrosis in patients with steatosis.[42] Because metformin is not metabolized via the hepatic CYP450 system, its pharmacokinetic characteristics do not expose patients to drug–drug interactions.[43] However, there have only been a few reported cases of the hepatotoxic side effects of metformin, but there may be an increased risk of developing lactic acidosis in the setting of diabetes-induced impaired liver function.[43–45] Therefore, the findings of the present study suggest that metformin treatment is not therapeutic in terms of reducing ALT levels in prediabetes-related NAFLD complications. However, the relationship between these liver enzymes and prediabetes may be more complex than generally appreciated. Future studies are warranted to help provide insight into the nature of these processes and determine the joint role of these liver enzymes in the pathophysiology of prediabetes.

Studies have reported that continuously low bilirubin levels are strongly correlated to the risk of development of diabetes-related cardiovascular complications.[16,46,47] Additionally, bilirubin has been reported to be inversely associated with NAFLD and steatohepatitis.[48] In this study, the induction of prediabetes resulted in a significant reduction of plasma total bilirubin concentration. However, the administration of the ruthenium complex re-

sulted in a significant increase in plasma bilirubin levels. We speculate that these findings may be due to the metal complex activating several key reactions, in particular those catalyzed by heme oxygenase 1, biliverdin reductase, and UDP-glucuronosyltransferase enzymes, which play an important role in bilirubin homeostasis.[47,49] Although we have not investigated these reactions in this study, this metal compound has been shown to have cardioprotective properties in diet-induced prediabetes rats and we speculate that these effects could be, in part, attributed to its effects on the synthesis of bilirubin in the liver.[24] Recent studies have identified bilirubin to be a major contributor to the total antioxidant capacity in blood.[47] Indeed, ruthenium(II) Schiff base complex has been shown to possess antioxidant ability as it reduced tissue lipid peroxidation and increased the concentration of antioxidant markers such as glutathione peroxidase and superoxide dismutase.[24] Metformin-treated animals also showed increased plasma total bilirubin when compared with both the nonprediabetic and the prediabetic control groups further explaining the reported cardioprotective effects of this compound.

Studies show that insulin resistance plays a key role in hepatic lipid accumulation and the subsequent increase of adipose tissue lipolysis.[50] The resultant fatty acid accumulation disturbs the β-oxidation system in the hepatic mitochondria and leads to further infiltration of fats in the liver.[50] In the liver, the molecular basis for this mechanism was shown to involve SREBP-1c, an important lipogenic transcription factor.[4,12,13] In the present study, the plasma level of SREBP-1c in the prediabetic control animals was significantly higher than that in the nonprediabetic control animals. The administration of the ruthenium(II) complex coupled with dietary intervention tended to reduce plasma SREBP-1c levels in prediabetic treated animals. Suggesting a potent effect of the metal in conjunction with dietary modification. Numerous Schiff base ruthenium(II) complexes have been isolated and their structural diversity have culminated into a wide spectrum of structure-activity of relationships.[25,51] For instance, the stereo-electronic features of the organic chelating ligands have been closely correlated to DNA-binding studies and cytotoxicity while the redox properties of the metal centre typically dictate their radical scavenging capabilities.[25,51,52] We speculate that the insulin-sensitizing effect of this compound may relate to the decreased SREBP-1c levels in ruthenium-treatment group. It might be, therefore speculated that administration of the ruthenium(II) complex in the presence of dietary intervention ameliorated the lipid accumulation in liver. Furthermore, decrease SREBP-1c levels were observed in the metformin-treated prediabetic rats. Metformin has been shown to activate AMP-activated protein kinase phosphorylation that inhibited SREBP-1c activity and attenuated hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice.[53] Indeed, histological analysis showed that the administration of the ruthenium(II) Schiff base complex resulted in reduced hepatic lipid droplet accumulation as well as reduced hepatocyte ballooning and lobular disarray by comparison to the prediabetic animals group, with the metformin-treated prediabetic rats with similar results. This possibly indicates that this compound restored the balance between hepatic lipid storage and removal thus resulting in a reduction in triacylglycerol accumulation.[50] Although more studies are needed to elucidate the mechanism by which this occurs, these results further suggest the ability of this compound to protect the liver against NAFLD.

Conclusions

The results of this study suggest that the administration of the ruthenium(II) Schiff base complex normalized the concentration of liver damage biomarker enzymes, significantly reduced hepatic lipid accumulation, decreased SREBP-1c plasma levels, and prevented the onset of hepatomegaly observed in prediabetic animals, although the underlying mechanisms are yet to be determined. This warrants further studies into this ruthenium(II) Schiff base complex as a potential therapeutic alternative for prediabetes associated NAFLD.

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L. P. Mabuza and M. W. Gamede were involved in study design, in vivo studies, data analysis, and assisted in preparing the manuscript. S. Maikoo and I. N. Booysen synthesized and spectroscopically characterized the free-ligand and its ruthenium complex, which was utilized as the metal-based drug. I. N. Booysen proofread the manuscript. A. Khathi and P. S. Ngubane were involved in conceptualization and design of the study, execution of animal studies, data analysis, provided funding, and assisted in writing the manuscript. All authors read and approved the manuscript.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.curtheres.2019.100570.

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