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S-allyl cysteine and Taurine attenuate diabetic nephropathy in rats via the inhibition of oxidative stress and recovering histopathological changes

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Abstract

Purpose – Renal failure is an end-stage consequence after persistent hyperglycemia during diabetic nephropathy (DN), and the etiology of DN has been linked to oxidative stress. The purpose of this research was to determine the beneficial synergistic effects of S-Allyl Cysteine (SAC) and Taurine (TAU) on oxidative damage in the kidneys of type 2 diabetic rats induced by hyperglycemia.

Design/methodology/approach – Experimental diabetes was developed by administering intraperitoneal single dose of streptozotocin (STZ; 65 mg/kg) with nicotinamide (NA; 230 mg/kg) in adult rats. Diabetic and control rats were treated with SAC (150 mg/kg), TAU (200 mg/kg) or SAC and TAU combination (75 + 100 mg/kg) for four weeks. The estimation of body weight, fasting blood glucose (FBG), oral glucose tolerance test (OGTT), oxidative stress markers along with kidney histopathology was done to investigate the antidiabetic potential of SAC/TAU in the NA/STZ diabetic group.

Findings – The following results were obtained for the therapeutic efficacy of SAC/TAU: decrease in blood glucose level, decreased level of thiobarbituric acid reactive substances (TBARS) and increased levels of GSH, glutathione-s-transferase (GST) and catalase (CAT). SAC/TAU significantly modulated diabetes-induced histological changes in the kidney of rats.

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Conflict of interest statement: There are no conflicts of interest declared by the authors.

Originality/value – SAC/TAU combination therapy modulated the oxidative stress markers in the kidney in diabetic rat model and also prevented oxidative damage as observed through histopathological findings.

Keywords Type 2 diabetes, Oxidative stress, Taurine, Glycemic control, S-allyl cysteine

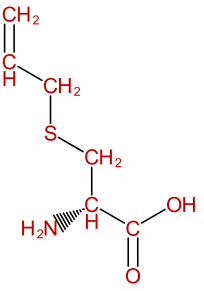
Paper type Research paper

Introduction

Diabetes mellitus (DM) is a fusion of metabolic abnormalities, described by deteriorating glycemic control and associated with several complications such as neuropathy, nephropathy, retinopathy, micro- and macro-vascular degradation (Maranta, Cianfanelli, & Cianflone, 2021; Unnikrishnan, Radha, & Mohan, 2021). The generation of reactive oxygen species (ROS) associated with chronic hyperglycemia, which causes oxidative stress, is a well-known and widely accepted mechanism in the development of diabetes and related complications (Deng *et al.*, 2021; Folli *et al.*, 2011; Ola, 2021). Previous investigations, both in experimental diabetic animals and in human diabetic patients, have shown elevated levels of oxidative stress with the inordinate formation of ROS and lower levels of the body's antioxidant network (Iacobini, Vitale, Pesce, Pugliese, & Menini, 2021; McMurray, Patten, & Harper, 2016; Volpe, Villar-Delfino, Dos Anjos, & Nogueira-Machado, 2018). The possible mechanisms of oxidative damage in diabetic nephropathy (DN) are hyperglycemia-induced activation of β-cell apoptotic pathways, deposition of advanced glycation end products (AGEs) and dysfunction of insulin synthesis (Chang *et al.*, 2021; Rodrigues *et al.*, 2014). Although other mechanisms are also involved, oxidative stress plays a crucial role, and it seems that these mechanisms are additional in causing the DN (Rodrigues *et al.*, 2014).

Thus, antioxidant supplementation is a viable strategy for preventing or reducing the negative consequences of diabetes. Treatment with exogenous or endogenous antioxidants has been examined and validated as a supplemental therapy for DM. Allium plants such as garlic (*Allium sativum*, Liliaceae) produce S-allyl cysteine (SAC; S-hydrocarbyl-L-cysteine) (Figure 1) (NCBI, 2022a), an organosulfur amino acid (Aziz, Ramalingam, Latip, & Zainalabidin, 2021). Aged garlic extract contains more durable and bioavailable water-soluble organosulfur components like S-allylmercapto-L-cysteine (SAMC), SAC, ajoene, alliin and allicin (Asdaq *et al.*, 2021; Baseggio Conrado, Fanelli, McGuire, & Ibbotson, 2021; Rais, Ved, Ahmad, & Parveen, 2021). In several studies, SAC (a phytochemical) has been documented to have antioxidant (Ruiz-Sanchez, Pedraza-Chaverri, Medina-Campos, Maldonado, & Rojas, 2020), antihepatotoxic (Anandasadagopan *et al.*, 2017), neurotrophic (Tobon-Velasco *et al.*, 2012) and anticancer (Xu *et al.*, 2018) activities. Some earlier studies have shown the antidiabetic effects of SAC in diabetic models due to its antioxidant potential (Saravanan and Ponmurugan, 2010, 2011, 2013; Zhai *et al.*, 2018).

On the other hand, Taurine (TAU; 2-aminoethane sulfonic acid) (Figure 2) (NCBI, 2022b) is also a sulfur-containing β-amino acid present in nearly all mammalian tissues and the most



Source(s): https://pubchem.ncbi.nlm.nih.gov/compound/S-allylcysteine

Figure 1.
Chemical structure of S-allyl cysteine (SAC)

ubiquitous free endogenous biomolecule in human cells. TAU makes up 0.1% of a normal human's total weight, or 70 g, in a 70 kg person (Jacobsen & Smith, 1968; Jong, Sandal, & Schaffer, 2021). TAU is produced in the body from methionine (an essential amino acid) and cysteine (a non-essential amino acid). There are three known pathways for the synthesis of TAU from cysteine. These synthesis pathways need pyridoxal-5'-phosphate (P5P), the functional coenzyme form of vitamin B6, as a co-factor. A vitamin B6 deficiency has been shown to impair TAU synthesis (Schaffer & Kim, 2018). TAU, unlike other amino acids, is not integrated into proteins and is found in abundance in numerous tissues, including cardiac and skeletal muscles, as well as the brain (Jong *et al.*, 2021). TAU is present in seafood (particularly shellfish like mussels, clams and oysters), muscle meat and organs (particularly the heart and liver) and dark flesh of chicken and turkey. TAU deficiency is a possibility for those who do not consume certain items on a regular basis, especially vegetarians (Rana & Sanders, 1986). In numerous studies, TAU has been reported to have hepatoprotective (Younis, Ghanim, Elmorsy, & Metwaly, 2021), antioxidant (Baliou *et al.*, 2021), nephroprotective (Madbouly, Azmy, Salama, & El-Amir, 2021), neuroprotective (Kumari, Prentice, & Wu, 2013; Silva *et al.*, 2021) and cardioprotective (Qaradakhi *et al.*, 2020; Samadi *et al.*, 2021) properties. Recently, the potential role of TAU to prevent diabetes and diabetes-related complications has been reviewed (Ito, Schaffer, & Azuma, 2012; Sarkar, Basak, Ghosh, Kundu, & Sil, 2017).

Despite the fact that SAC and TAU have various pharmacological and therapeutical properties in combating several metabolic disorders and diabetes related complications, their combined beneficial effects against type 2 diabetes mellitus (T2DM) in nicotinamide (NA)/streptozotocin (STZ)-induced model had not been explored. The current study is a step ahead to explore the anti-diabetic consequences of the combined action of SAC and TAU in NA/STZ-induced diabetic rats.

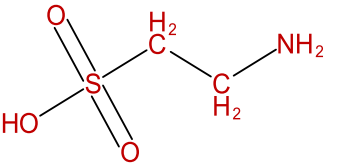
Materials and methods

Chemicals and reagents

Reduced glutathione (GSH) (70-18-8), oxidized GSH (27025-41-8), STZ (18883-66-4), NA (98-92-0), thiobarbituric acid (TBA) (504-17-6), trichloroacetic acid (TCA) (76-03-9), ethylene diamine tetra acetic acid (EDTA) (60-00-4), nicotinamide adenine dinucleotide phosphate (NADPH) (24292-60-2), 1-chloro-2,4-dinitrobenzene (CDNB) (97-00-7), phenylmethylsulfonyl fluoride (PMSF) (329-98-6), ethylene glycol tetraacetic acid (EGTA) (67-42-5) and 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (69-78-3) were procured from Sigma-Aldrich Chemicals (Ltd.), Delhi, India. SAC (21593-77-1) and TAU (107-35-7) were procured from LGC-Prochem (Ltd.), Bangalore, India. Bovine serum albumin (BSA) (9048-46-8) and sulfosalicylic acid (SSA) (5965-83-3) were purchased from SRL Chemicals (Ltd.), Mumbai, India. The rest of the chemicals were of the analytical reagent grade.

Animals

All of the studies were carried out on male Wistar rats (160–200g). Prior to dietary manipulation, these rats were given a conventional rodent pellet diet from Hindustan Lever



Source(s): https://pubchem.ncbi.nlm.nih.gov/compound/Taurine

Figure 2.
Chemical structure of Taurine (TAU)

(Ltd.), Mumbai, India and water *ad libitum*. All methods for handling and utilizing the animals were assessed and authorized by the Institutional Animal Ethical Committee (VCTE/IAEC/155), which is recognized by the Committee for the Purpose of Control and Supervision on Experiments on Animals (1446/PO/Re/S/11/CPCSEA), Chennai, India.

Development of type 2 diabetes model

Experimental T2DM was developed by administering NA and STZ in adult rats. The animals (fasted overnight) received an intraperitoneal NA (230 mg/kg in saline), 15 minutes before the intraperitoneal ingestion of STZ (65 mg/kg), diluted in 0.1 M ice-cold citrated buffer (pH 4.5) instantly before usage. After the administration of NA/STZ, the animals were permitted standardized food and water access *ad libitum*. Blood glucose was evaluated after two days, and the animals with glucose level ≈140 ± 8 mg/dl were categorized as diabetics and recruited for further investigation (Masiello *et al.*, 1998; Rais *et al.*, 2023).

Mechanism of T2DM

It is generally known that STZ has the ability to cause DM because of its severe cytotoxic effects on β-cells, which are similar to the pathophysiology of T1DM. Masiello, an Italian scientist, developed a truly valuable T2DM model of NA/STZ in 1998, which is based on the ability of NA to assert defensive effects over the β-cytotoxic consequences of STZ (Masiello *et al.*, 1998). The genotoxic nature of STZ in animals is achieved through a diminution of nicotinamide adenine dinucleotide (NAD^+) in the pancreatic β-cells via the transmembrane carrier protein known as glucose transporter 2 (GLUT2) that can induce cell damage through DNA strand breakdowns, leading to apoptosis. Excessive DNA damage contributes to the overactivation of poly-ADP-ribose-polymerase-1 (PARP-1), loss of cellular resources and necrotic cells death. NA is a PARP-1 inhibitor and also a metabolic precursor of NAD^+. NAD^+ is a significant redox reaction coenzyme that is essential for the production of adenosine triphosphate (ATP) as well as several other metabolic processes. Therefore, some of the pancreatic β-cells remain unharmed by administering NA and are capable of secreting insulin to induce a model of T2DM (Masiello, 2006; Masiello *et al.*, 1998; Rais *et al.*, 2023).

Experimental design

Following the successful induction of T2DM, animals were arbitrarily separated into five groups, each with eight animals ($n = 8$). After this strategic segregation, each group of animals was dosed with a different regimen of treating molecules on a daily basis for a period of 30 days. For this, we used oral normal saline; SAC (150 mg/kg, b.w.) (Abdi, Afjal, Najmi, & Raisuddin, 2018; Sathibabu Uddandrao *et al.*, 2019; Uddandrao *et al.*, 2020) TAU (200 mg/kg, b.w.) (Ahmadi & Mehranjani, 2021; Bhattacharjee, Prajapati, & Krishnamurthy, 2021; Heidari, Jamshidzadeh, Ghanbarinejad, Ommati, & Niknahad, 2018); SAC/TAU combination (75 + 100 mg/kg, b.w.) (Rais *et al.*, 2023) and glibenclamide (GL; 10 mg/kg, b.w.) as a standard drug (He *et al.*, 2021; Nguyen, Pham, Luong, Le, & Vo, 2020) as five treatment regimens were given separately to five groups of animals. For appropriate comparison, two separate nondiabetic groups were designed to receive normal saline and SAC/TAU combination as controls.

Blood sampling and tissue preparation

Blood was drawn retro-orbitally from the internal ocular canthus using haematocrit microcapillaries and subjected to the biochemical analysis of conventional indices of hyperglycemia. At the culmination of the treatment, rats were euthanized by neck-cervical displacement, and their kidneys were removed and then imbued with ice-cold saline immediately. To avoid *ex vivo* oxidation or auto-oxidation of the tissues, homogenization was performed at 4°C

with 10 times (w/v) in 0.1M phosphate-buffer (pH 7.4) comprising inhibitors of protease: 1.5 mM aprotinin, 0.04% butylated hydroxytoluene, 5 mM leupeptin, 1 mM benzamidine, 10 mM EDTA, 3 mM peptastatin A, 2 mM PMSF and 0.1 mM EGTA. To carve-up the cellular debris, the homogenate was centrifuged at 800 × g for 5 min at 4°C and used for thiobarbituric reactive substance quantification. The post-mitochondrial supernatant (PMS) was obtained by centrifuging the supernatant again at 10,000 × g for 20 minutes at 4°C, which was employed in a variety of biochemical procedures (Parveen, Ishrat, Malik, Kausar, & Siddiqui, 2013).

Analytical procedures

Body weight changes

The initial and final (before treatment and after treatment respectively) body weights of the animals were documented at every week between 9.00 and 10.00 am for four weeks to analyze the changes in diabetic and treated groups.

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was conducted to evaluate the variations in glucose tolerance in the final week of the experiment. For this study, overnight starved rats were administered with glucose (2 g/kg body weight) by mouth. Blood was taken from the orbital sinus at 0-, 30-, 60-, and 120-min frequencies for glucose assessment. This operation was performed without the use of anesthesia on the animals (Parveen *et al.*, 2013).

Blood glucose estimation

The glucose oxidase/peroxidase (GOD/POD) method was used to estimate the fasting blood glucose (FBG) levels employing a commercially available assessment tool from Span Divergent Limited, Surat, India. According to the instructions and guidance provided by the kit, the preparation and process were carried out (Trinder, 1969).

Oxidative stress markers

Thiobarbituric acid reactive substances (TBARS) content. The methodology of Utley, Bernheim, and Hochstein (1967) with slight adjustments was performed to evaluate the amount of lipid peroxidation (LPO). The homogenate (0.25 ml) was poured into 15 × 100-mm test tubes and kept for incubation in a metabolic shaker for one hour at 37°C. An equivalent proportion of the homogenate was poured into centrifugal tubes, which were kept at 0°C and labeled as zero-hour incubation. Upon one hour of incubation, each test tube was filled with 0.5 ml of 5% frigid TCA (w/v) proceeded by 0.5 ml of 0.67 % TBA (w/v) then centrifuged at 1,000 × g for 15 min. After that, the supernatant was taken to different test tubes and heated in a hot water bath for 10 min. The resulting pink tinted absorbance was recorded at 535 nm in a spectrophotometer (Shimadzu-UV-1601, Japan). The thiobarbituric acid reactive substances (TBARS) value was estimated by applying a molar absorptivity extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$ and represented as of TBARS formed nmol/min/mg of protein (Utley *et al.*, 1967).

Assay for reduced glutathione (GSH). The method of Jollow, Mitchell, Zampaglione, and Gillette (1974) with minor modifications was performed to determine the reduced glutathione (GSH) content. PMS was blended with 4.0% sulfosalicylic acid (w/v) in a ratio of 1:1 (v/v). The samples were incubated for one hour at 4°C before being centrifuged at 1,200 × g for 15 min at 4°C. In a total quantity of 1.0 ml, the assay miscellany comprised 0.1 ml of supernatant, 0.1M phosphate buffer (pH 7.4), and 1.0 mM DTNB. A yellow color was generated and recorded instantly at 412 nm in a spectrophotometer (Shimadzu-UV-1601, Japan). The GSH proportion was estimated by applying a molar extinction coefficient value of $13.6 \times 103\ M^{-1}\ cm^{-1}$ and represented as DTNB mmol/mg of protein (Jollow *et al.*, 1974).

Assay for glutathione-s-transferase (GST). The method of Habig, Pabst, and Jakoby (1974) with slight modifications was performed to measure the behavior of glutathione-s-transferase (GST). In a total proportion of 3.0 ml, the reaction mixture comprised 1.0 mM CDNB, 0.1 ml PMS, 1.0 mM GSH and 0.1 M phosphate buffer (pH 7.4). The variations in absorbance were documented at 340 nm by using spectrophotometer (Shimadzu-UV-1601, Japan), and enzyme performance was estimated by applying a molar extinction coefficient value of $9.6 \times 103\ M^{-1}\ cm^{-1}$ and represented as nmol of CDNB conjugate formed per min per mg of protein (Habig *et al.*, 1974).

Assay for catalase (CAT). The methodology of A. Claiborne (1985) with slight modifications was employed to measure the activity of catalase (CAT). Concisely, in a total proportion of 3.0 ml, the assay mixture comprised 0.05 ml PMS, 0.019 M hydrogen peroxide (H_2O_2) and 0.05 M phosphate buffer (pH 7.0). The variations in absorbance were measured at 240 nm, and the activity of catalase was represented as nmol H_2O_2 consumed per min per mg of protein (Claiborne, 1985).

Histopathology

In order to conduct histological evaluations, hematoxylin (H) and eosin (E) were employed to stain kidneys from various groups. Concisely, the rats were anesthetized and transcardially distended with saline at the end of the experiment. Kidneys were removed instantly and postfixed in 10% buffered formalin for 24 hours. Thin slices (3–4 mm) of kidney tissues were dried and engrafted in paraffin after the fixing process was completed. Each kidney was dissected into at least four cross-sections of 5-µm thickness and stained with H and E dyes. Dibutyl phthalate xylene (DPX) was used to mount each tissue segment (Carrara *et al.*, 2020). The slides were examined for histopathological alterations, and magnified microscopic photographs were obtained by using a microscopic equipment (Olympus BX-50, Japan).

Statistical analysis

The data were stated as mean ± SEM ($n = 8$). The results were statistically analyzed by using SPSS-16 software and applying ANOVA proceeded by Tukey's post-hoc procedure for biochemical parameters. A *p-value* of less than 0.05 was noted statistically significant.

Results

The effect of SAC, TAU and SAC/TAU on body weight in the NA/STZ-induced rat model of diabetes

The body weights (initial and final) of all animal groups were documented separately (Table 1). The study conclusively depicted that the group of control rats acquired body

S. No.	Groups	Body weight (gm) Initial	Final
1	Control (C)	185.6 ± 6.0	214.3 ± 5.5
2	C + SAC/TAU	182 ± 5.5	212 ± 3.5
3	NA/STZ	191.6 ± 8.0*	165 ± 6.0*
4	NA/STZ + SAC	194 ± 8.0**	189 ± 6.5**
5	NA/STZ + TAU	195 ± 6.0**	185 ± 7.5**
6	NA/STZ + SAC/TAU	196 ± 7.0***	203 ± 4.0***
7	NA/STZ + GL	192 ± 5.5***	186 ± 3.5***

Note(s): NA/STZ group demonstrated considerable changes in body weight in contrast with the controlled group (*$p < 0.05$ NA/STZ vs control group). SAC, TAU, SAC/TAU and GL treatment significantly ameliorated body weight when tried to compare with the NA/STZ group (**$p < 0.05$ NA/STZ vs NA/STZ + SAC or NA/STZ + TAU and ***$p < 0.05$ NA/STZ vs NA/STZ + SAC/TAU or NA/STZ + GL group). Values are expressed as mean ± SEM ($n = 8$)

Table 1. The effect of SAC, TAU and SAC/TAU treatment on body weight of control and experimental groups

weight (mean ± SEM) from 185.6 ± 6.0g on the first day to 214.3 ± 5.5g on the final day of the study.

During the same period of treatment, the diabetic group (NA/STZ) of rats had shown a move in body weights from a value (mean ± SEM) of 191.6 ± 8.0g on the first day to 165 ± 6.0g on the final day of the study. These differences in the body weights illustrated that the diabetic rats showed a gradual fall in body weight that was reported to be significant ($p < 0.05$) throughout the treatment phase as against the weight gain seen in the control group of rats.

The SAC/TAU treated group of control rats showed body weights (mean ± SEM) of 182 ± 5.5g on the first day to 212 ± 3.5g on the final day of the study, and it was found that there was an elevation in body weight at the last day of the study. SAC, TAU, SAC/TAU and GL treated groups of diabetic rats showed body weights (mean ± SEM) of 194 ± 8.0g to 189 ± 6.5g, 195 ± 6.0g to 185 ± 7.5g, 196 ± 7.0g to 203 ± 4.0g and 192 ± 5.5g to 186 ± 3.5g, respectively, from the first day to final day of the study. It was concluded that administration of SAC, TAU, SAC/TAU and GL restored body weight significantly ($p < 0.05$) when compared with the NA/STZ-induced group.

SAC/TAU treatment ameliorated OGTT in the NA/STZ-induced rat model of diabetes

After oral ingestion of glucose (2g/kg), the blood glucose levels of rats in all groups were measured at various time points (0-, 30-, 60- and 120-min). Readings of glucose monitoring are illustrated in Figure 3.

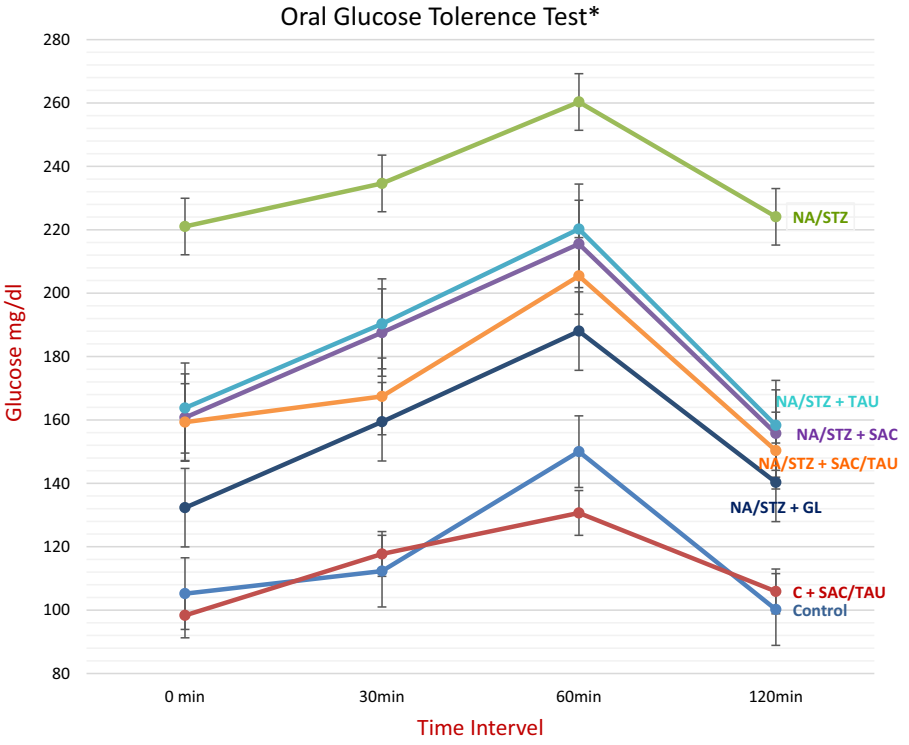
The maximal elevation in blood glucose level was detected in the NA/STZ group after 60 min and stayed high for the next 60 min. The NA/STZ + SAC/TAU group exhibited significant ($p < 0.05$) reduction in blood glucose levels at 60 and 120 min when compared with the NA/STZ group, NA/STZ + SAC group and NA/STZ + TAU group.

The effect of SAC, TAU and SAC/TAU supplementation on hyperglycaemia in the NA/STZ-induced diabetic rat model

The FBG levels of various categories of rats were recorded throughout the dose treatment phase of the investigation (Figure 4). According to the current study, data showed that the overnight FBG value (mean ± SEM) of the control group of rats was 123.29 ± 0.71 mg/dl. SAC/TAU treated group of control rats showed an FBG level (mean ± SEM) of 119.67 ± 1.31 mg/dl. It was observed that the FBG levels of the control animals given no medication and the group of control rats given SAC/TAU treatment did not significantly differ from each other. NA/STZ treated group showed an FBG level (mean ± SEM) of 263.31 ± 0.89 mg/dl. SAC, TAU, SAC/TAU and GL treated group of type 2 DM rats showed FBG levels (mean ± SEM) of 171.72 ± 0.39 mg/dl, 182.13 ± 0.71 mg/dl, 132.17 ± 1.54 mg/dl and 126.23 ± 1.12 mg/dl, respectively. The four weeks treatment with SAC and TAU in combined form reflected in significant ($p < 0.05$) hypoglycaemic impact and was comparable to standard glibenclamide.

Effect of SAC, TAU and SAC/TAU supplementation on TBARS contents in the NA/STZ-induced diabetic rat model

The TBARS levels of the different groups of animals were recorded during the treatment period (Figure 5). Data showed that the TBARS level (mean ± SEM) of the normal group of rats was 1.93 ± 0.173. While the SAC/TAU treated group of control rats showed a TBARS level (mean ± SEM) of 1.94 ± 0.159. On the contrary, the NA/STZ-induced group showed a TBARS level (mean ± SEM) of 6.02 ± 0.242, which was reported to be significant ($p < 0.05$) in comparison to the TBARS level seen in both control groups of rats.



Note(s): After 60 minutes, the NA/STZ diabetic group showed the greatest spike in blood glucose levels, which persisted for the following 60 minutes. The NA/STZ + SAC/TAU group exhibited notable ($p < 0.05$) diminution in blood glucose levels at 60 and 120 minutes in contrast to the NA/STZ group, NA/STZ + SAC group, and NA/STZ + TAU group. Values are expressed as mean ± S.E.M. ($n = 8$)

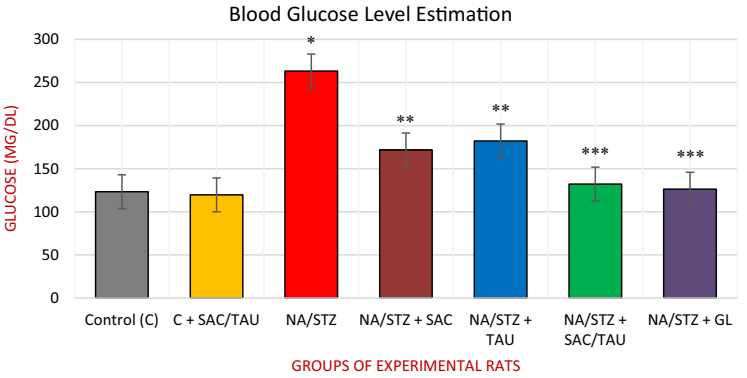
Figure 3.
The effect of SAC, TAU and SAC/TAU treatment on oral glucose tolerance test

SAC, TAU, SAC/TAU and GL administered group of diabetic rats showed a TBARS levels (mean ± SEM) of 3.93 ± 0.148, 3.85 ± 0.178, 3.04 ± 0.169 and 3.18 ± 0.149, respectively. The study conclusively depicted that TBARS level was not significantly changed in SAC/TAU treated control group as compared to untreated control group. The diabetic rats in the NA/STZ group had significantly ($p < 0.05$) higher levels of TBARS as compared with normal control rats. The level of TBARS dropped significantly ($p < 0.05$) in the NA/STZ induced group that received GL or SAC or TAU or SAC/TAU treatment in comparison to the diabetic group.

The combined dose of SAC and TAU showed a significant ($p < 0.05$) decrease in TBARS level in the NA/STZ-induced group of rats in comparison to the separate treatment with SAC and TAU.

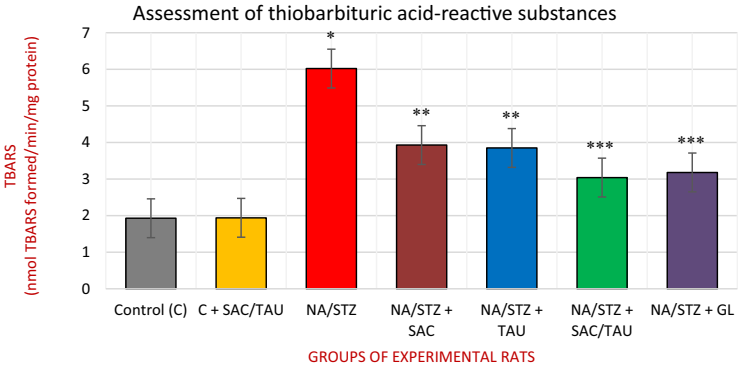
The effect of SAC, TAU and SAC/TAU supplementation on GSH in the NA/STZ-induced diabetic rat model

The GSH levels of the different groups of animals during the treatment period were recorded (Figure 6). Data showed that the GSH level (mean ± SEM) of the normal control group of rats



Note(s): NA/STZ group indicated a remarkable elevation in FBG level when contrasted with the controlled group (*$p < 0.05$ NA/STZ vs. control group). SAC, TAU, SAC/TAU combination and GL treatment notably ameliorated the increased FBG level when contrasted with the NA/STZ group (**$p < 0.05$ NA/STZ vs. NA/STZ + SAC or NA/STZ + TAU and ***$p < 0.05$ NA/STZ vs. NA/STZ + SAC/TAU or NA/STZ + GL group)

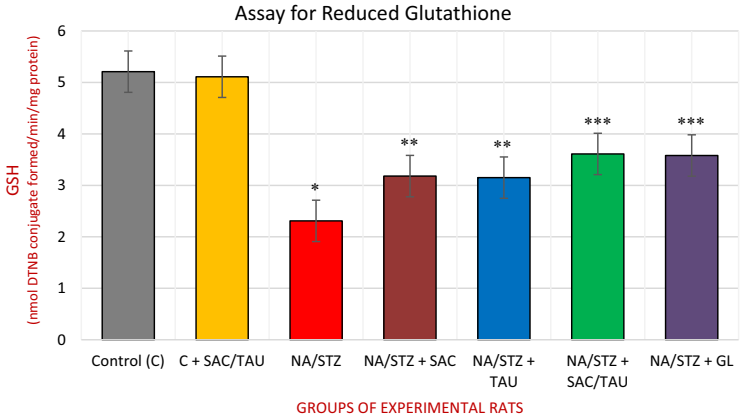
Figure 4.
The effect of SAC, TAU and SAC/TAU treatment on FBG



Note(s): NA/STZ group demonstrated a remarkable augmentation in TBARS levels in contrast with the controlled group (*$p < 0.05$ NA/STZ vs. control category). SAC, TAU, SAC/TAU combination, and GL treatment significantly reduced TBARS when contrasted with the NA/STZ category (**$p < 0.05$ NA/STZ vs. NA/STZ + SAC or NA/STZ + TAU and ***$p < 0.05$ NA/STZ + SAC/TAU or NA/STZ + GL category). Values are expressed as mean ± S.E.M. ($n = 8$)

Figure 5.
The effect of SAC, TAU and SAC/TAU on TBARS contents

was 5.21 ± 0.198. The SAC/TAU administered group of control rats showed a level (mean ± SEM) of 5.11 ± 0.216. The NA/STZ induced group showed GSH level (mean ± SEM) of 2.31 ± 0.155 which was documented to be significant ($p < 0.05$) as compared to GSH level seen in the control group of rats. SAC, TAU, SAC/TAU and GL treated groups of diabetic rats showed GSH levels (mean ± SEM) of 3.18 ± 0.199, 3.15 ± 0.243, 3.61 ± 0.163 and 3.58 ± 0.201, respectively.



Note(s): NA/STZ group exhibited a remarkable diminution in GSH when contrasted with the controlled group (*$p < 0.05$ NA/STZ vs. control group). SAC, TAU, SAC/TAU combination and GL treatment significantly restored GSH when equated with the NA/STZ category (**$p < 0.05$ NA/STZ vs. NA/STZ + SAC or NA/STZ + TAU and ***$p < 0.05$ NA/STZ vs. NA/STZ + SAC/TAU or NA/STZ + GL category). Values are expressed as mean ± S.E.M. ($n = 8$)

Figure 6.
The effect of SAC, TAU and SAC/TAU on GSH

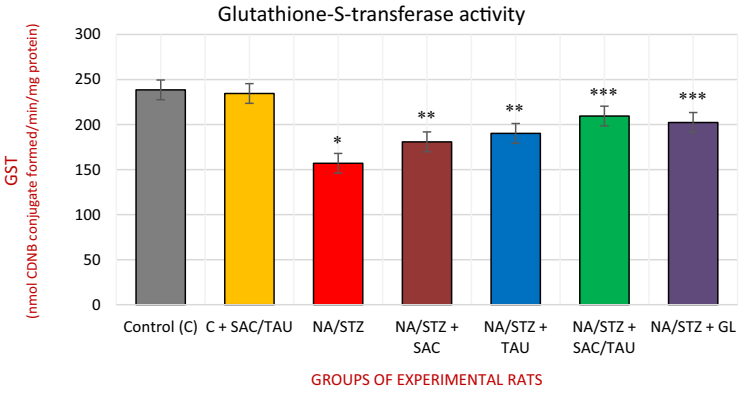
Thus, the study conclusively depicted that GSH level in SAC/TAU treated control group was not significantly changed as compared to the control group. Nevertheless, a significant ($p < 0.05$) reduction in GSH was recorded in the NA/STZ-induced diabetic group when compared with the control group. GSH level increased significantly ($p < 0.05$) in those groups of rats which received GL or SAC or TAU or SAC/TAU treatment in comparison to the diabetic group. The combined dose of SAC and TAU showed a significant ($p < 0.05$) increase in GSH level in the NA/STZ induced group of rats in comparison to the separate treatment with SAC and TAU.

The effect of SAC, TAU and SAC/TAU supplementation on GST and CAT activity in the NA/STZ-induced diabetic rat model

The activities of GST (Figure 7) and CAT (Figure 8) of separate groups of animals during the treatment phase were recorded. Data showed that the values (mean ± SEM) of GST and CAT of the normal group of rats were 238.53 ± 0.143 and 94.53 ± 0.372, respectively. The SAC/TAU treated group of control rats showed activities values of GST and CAT of 234.52 ± 0.377 and 92.65 ± 0.232, respectively.

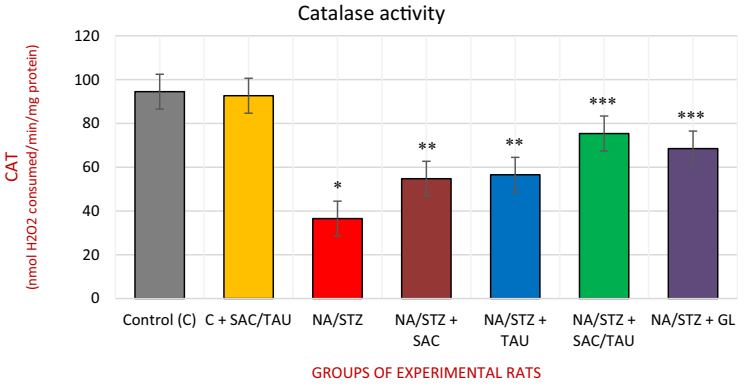
The GST and CAT activities values (mean ± SEM) in the NA/STZ-induced group were monitored as 156.98 ± 0.212 and 36.54 ± 0.319, respectively, which was significantly low ($p < 0.05$) as compared to those of the control group of rats. On the contrary, SAC, TAU, SAC/TAU and GL treated groups of diabetic rats showed GST activity values (mean ± SEM) of 180.83 ± 0.267, 190.18 ± 0.199, 209.48 ± 0.223 and 202.33 ± 0.198, respectively. While CAT activity values (mean ± SEM) in SAC, TAU, SAC/TAU and GL treated group of diabetic rats showed of 54.76 ± 0.463, 56.55 ± 0.226, 75.4 ± 0.293 and 68.53 ± 0.401, respectively.

The study conclusively depicted that activities of GST and CAT were not significantly changed in the SAC/TAU treated group of control as compared to the normal control group.



Note(s): NA/STZ group exhibited a remarkable decrement in GST activity as contrasted with the controlled category (*$p < 0.05$ NA/STZ vs. control group). SAC, TAU, SAC/TAU combination and GL treatment significantly increased GST activity when equated with the NA/STZ category (**$p < 0.05$ NA/STZ vs. NA/STZ + SAC or NA/STZ + TAU and ***$p < 0.05$ NA/STZ vs. NA/STZ + SAC/TAU or NA/STZ + GL group). Values are expressed as mean ± S.E.M. ($n = 8$)

Figure 7.
The effect of SAC, TAU and SAC/TAU on GST activity



Note(s): NA/STZ group depicted a remarkable decrement in CAT activity as contrasted with the controlled category (*$p < 0.05$ NA/STZ vs. control group). SAC, TAU, SAC/TAU combination and GL treatment notably augmented CAT activity when contrasted with the NA/STZ category (**$p < 0.05$ NA/STZ vs. NA/STZ + SAC or NA/STZ + TAU and ***$p < 0.05$ NA/STZ vs. NA/STZ + SAC/TAU or NA/STZ + GL group). Values are expressed as mean ± S.E.M. ($n = 8$)

Figure 8.
The effect of SAC, TAU and SAC/TAU on CAT activity

However, a significant ($p < 0.05$) reduction in the activities of GST and CAT was documented in the NA/STZ induced group compared to both control groups. The SAC or TAU treated diabetic rats showed significant ($p < 0.05$) increase in GST and CAT activities. The diabetic rats who received GL treatment also showed increased GST and CAT activities. Moreover,

supplementation with SAC and TAU in combination significantly ($p < 0.05$) augmented the activities of GST and CAT toward the normal control group comparing separate therapy.

Histopathological findings

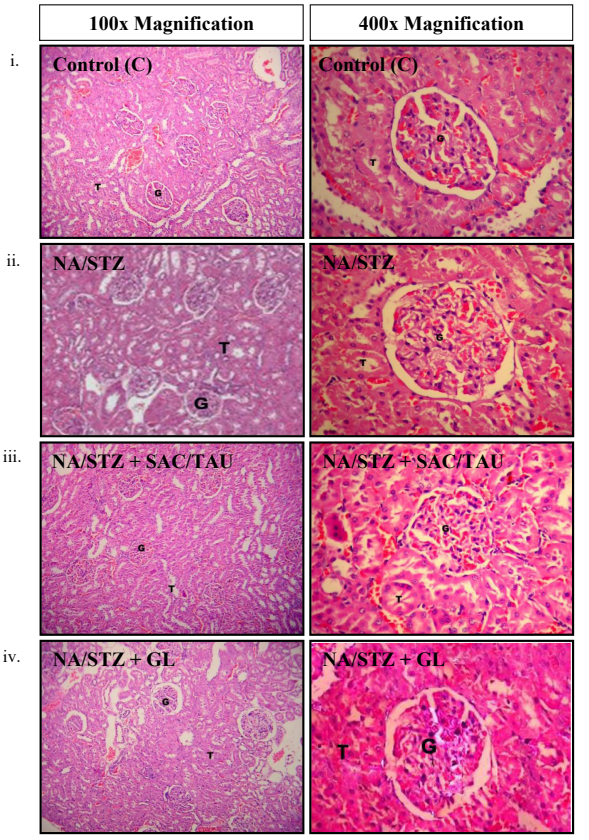
The effect of SAC and TAU combination therapy on the kidneys of NA/STZ-induced diabetic model of rats. A histological examination of renal tissues from different groups of rats was carried out with H&E staining. In both normal and pathological situations, H and E staining was employed to detect and identify tissue components (Figure 9). Histological microscopy exhibited the regular architecture of renal tubular epithelial cells with a glomerulus of normal size and cellularity. Tubules were within the normal limits. Kidney sections of the NA/STZ-induced group consistently evidenced damage to renal tubular epithelial cells and glomerulus with increased mesangium and thickened basement membranes. The severity of degenerative alterations was attenuated by SAC/TAU and GL supplementation in the NA/STZ + SAC/TAU and NA/STZ + GL group as compared with the NA/STZ-induced diabetic group denoting partial protective effects against the nephrotoxicity. The study conclusively depicted that the tested drug combination (SAC/TAU) exhibited protective potential against DN seen in the NA/STZ-induced model of rats.

Discussion

In our previous in vitro study, SAC and TAU combination was reported to have strong antioxidant and antidiabetic properties, indicating its potential for treating hyperglycemia and related complications (Rais, Ved, Ahmad, Parveen, & Mujeeb, 2021). According to an in vivo study, giving TAU and SAC-derivative (N-acetylcysteine) to male Sprague Dawley rats restored metabolic markers as a result of their strong antioxidant properties, which they exhibited through preventing oxidative reactions (Haber *et al.*, 2003). The current study showed that NA/STZ-induced hyperglycemia in rats is associated with oxidative deterioration in the kidneys. Furthermore, treatment with SAC and TAU in the combined form, by the merit of their antioxidant property, mitigated the NA/STZ-induced morphological and biochemical changes in rat kidneys. Indeed, SAC and TAU possess a vast ethno-medical history of therapeutic value; SAC and TAU have been proven to be used as protective agents in various disease conditions.

SAC has been demonstrated to have antioxidant (Khajevand-Khazaei *et al.*, 2019), anticancer (Xu *et al.*, 2018), antihepatotoxic (Anandasadagopan *et al.*, 2017), antidiabetic (Saravanan & Ponmurugan, 2010, 2011, 2013, Zhai *et al.*, 2018) and neurotrophic activity (Kosuge, 2020; Tobon-Velasco *et al.*, 2012) while TAU has been confirmed to be exhibiting a wide spectrum of actions on cardiovascular complications and neuroprotection (Qaradakhi *et al.*, 2020; Samadi *et al.*, 2021). Nonetheless, TAU is also involved in the restoration of renal function in the presence of osmotic stress (Madbouly *et al.*, 2021). It was observed that the exogenous TAU prolonged the aging phase of tissues by means of its free radical scavenging properties (Baliou *et al.*, 2021). TAU has been shown to prevent the production of AGEs through its antioxidant potential in diabetes (Esmaeili, Maleki, Kheirouri, & Alizadeh, 2021; Nandhini, Thirunavukkarasu, & Anuradha, 2004).

Diabetes patients' bodies are unable to transport blood glucose for utilization as energy by their cells because of low insulin levels. When this happens, the body begins utilizing both adipose and muscular tissues as a source of fuel, which initiates to a decrease in total body weight (Halali *et al.*, 2022). Weight loss is a general characteristic of diabetes progression and could be considered an indirect index of consequences. Therefore, the body weights of all animal groups (initial and final) were recorded individually (Table 1). This primary assessment concluded that administration of SAC, TAU, SAC/TAU and GL restored body weight significantly ($p < 0.05$) when they were compared with the NA/STZ group.



Note(s): (i) The portrait photographs from the Control group at low magnification (100x) demonstrating a regular renal parenchyma, and at a high magnification (400x) showing glomerulus (G) with regular size and cellularity. Tubules (T) are within the acceptable ranges. (ii) NA/STZ treated group at low magnification (100x) showing the impairment to glomerulus. Tubular epithelial cells appear normal. At high magnification (400x) showing the glomerulus (G) with increased mesangium and thickened basement membranes. Tubules (T) are within the acceptable limits. (iii) SAC/TAU ingestion in the NA/STZ + SAC/TAU group at low magnification (100x) showing regular renal parenchyma, and at high magnification (400x) showing the glomerulus (G) with a mild increase in mesangium and no thickening of basement membranes. Tubules (T) are within normal limits. (iv) GL supplementation in the NA/STZ + GL group at low magnification (100x) showing usual histological morphology of renal cortex; at high magnification (400x) showing the normal glomerulus (G) and tubular (T) epithelial cells

Figure 9.
Kidney histopathological analysis of SAC, TAU and SAC/TAU combination

Glucose intolerance, a symptom of beta-cell malfunction, and decreased glucose-triggered insulin production are the characteristics of type 2 DM. Glucose intolerance is brought about by impaired insulin action in peripheral metabolic target tissues (Dalgard, Moller, & Kyvik, 2020). In clinical settings and in scientific investigations, the glucose tolerance test is used to identify people who have observable type 2 DM and deteriorated glucose tolerance. Due to its ease of application, it is the physiological test that is most frequently used for rodents to firstly examine their glucose homeostasis. When a bolus of glucose is administered, the OGTT monitors alterations in blood glucose values over a two-hour period (Nelson, 1988; Tan *et al.*, 2021).

In the current study, the diabetic group showed a hyperglycemic condition with a substantial elevation in blood glucose levels at 120 min post glucose administration in OGTT. On the contrary, SAC and TAU treatment reduced the elevated blood glucose level in NA/STZ group as represented by drop in peak blood glucose levels at 60 and 120 min during OGTT, thereby showing its antihyperglycemic activity. Adding to it, some histological, morphological and biochemical studies showed that TAU is an effective hypoglycemic agent, which had a protective and preserving essence on pancreatic β-cells (Gavrovskaya, Ryzhova, Safonova, Matveev, & Sapronov, 2008).

Excessive hyperglycemia results in glomerular capillary wall thickening which causes glomerular hypertension and hyperfiltration that leads to glomerulosclerosis, glomerular dysfunction and damage (Lee, Yang, Han, Choi, & Kim, 2019). The results of the present study illustrated that NA/STZ caused hyperglycaemia which was reported to be remarkable ($p < 0.05$) during the therapy period in contrast with FBG level seen in normal group of rats. The study conclusively depicted that the combined dose of SAC and TAU was more effective in lowering FBG levels in type 2 DM rats in contrast to the separate therapy with SAC and TAU.

Hyperglycemia activates the ROS formation which causes autooxidation of lipid membrane, modifying the trans-bilayer fluidity slope, inhibiting the processes of membrane-bound enzymes and receptors and thus leads to LPO. The overall consequence of it is the production of TBARS that leads to a loss of membrane stability, a key element in diabetes progression (Ahmad & Tahir, 2016; Deng *et al.*, 2021; Ola, 2021; Su *et al.*, 2019). TBARS are generated as a by-product of lipid molecules' peroxidation (i.e. as breakdown products of fats). Higher levels of TBARS indicate increased oxalate toxicity brought on by increased LPO (Lovell, Ehmann, Butler, & Markesbery, 1995).

It has been demonstrated that persistent oxidative stress conditions reduce the actions of antioxidant enzymes (such as GST and CAT) in diabetic rats (Salazar-Garcia & Corona, 2021). Enhanced oxidative stress can modify the redox capacity of GSH due to its thiol group oxidation (Salazar-Garcia & Corona, 2021; Ulrich & Jakob, 2019). Since GSH is needed as a substrate for the activity of GST, a depletion in GSH level may also decrease the antioxidant action of the enzyme GST. CAT is a vigorous scavenging enzyme that eliminates free radicals *in vivo*. Reduced activity can result in an excess of hydrogen peroxide (H_2O_2), which generates hydroxyl radicals ($^{\bullet}OH$), leading to genesis and proliferation of LPO. H_2O_2 is converted to H_2O by catalase or glutathione peroxidase (Gorny *et al.*, 2020; Hemerkova & Valis, 2021).

In the present experiment, we found that diabetic rats' kidneys had higher levels of TBARS (as a dynamic index of LPO rate) and lower levels of GSH and antioxidant enzymes (GST and CAT) activity. SAC and TAU treatment significantly reduced lipid and protein oxidation by increasing GSH levels and antioxidant enzyme status in the SAC/TAU treated group. The combination of SAC and TAU was more beneficial on antioxidant parameters than alone. The antioxidant efficiency of SAC/TAU was found to be more pronounced than GL. These findings suggest that SAC and TAU can scavenge or prevent free radical generation, as well as contribute to the stabilization of the endogenous antioxidant network, comprising GSH and reduce LPO in numerous free radical-induced situations, which is consistent with earlier studies (Deng *et al.*, 2021; Liu *et al.*, 2004; Pietta, 2000). Niu *et al.* have shown the increase in the protective effect of TAU against oxidative stress, which further contributed to its antioxidant potential (Niu, Zheng, Liu, & Li, 2018).

Histopathological observations further confirm our findings. The H and E-stained control kidneys exhibited regular morphology of renal tubular epithelial cells, as well as a glomerulus with normal size and cellularity. Tubules were observed to be within standard limits as well. Kidney sections of the NA/STZ induced group exhibited glomerulus with increased mesangium and thickened basement membranes. Tubules were within normal limits. SAC/TAU and GL supplementation reduced the intensity of deleterious effects in the NA/STZ + SAC/TAU and NA/STZ + GL treated group compared to the NA/STZ-induced group, demonstrating that SAC/TAU had a partial defensive effect against nephrotoxicity. Treated group shows the protective effect of the test drug against DN seen in the NA/STZ model consistent with previous studies (Huang, Chuang, Guh, Yang, & Hsu, 2008).

SAC has been proven to reduce oxidative stress by trapping ROS such as $O_2^{\bullet -}$, H_2O_2, $^{\bullet}OH$, $^{\bullet}NO$ and $ONOO^-$ radicals. SAC also inhibits the generation of glycation end products and glycation-derived free radicals (Ahmad, Pischetsrieder, & Ahmed, 2007; Colin-Gonzalez, Ali, Tunez, & Santamaria, 2015). Chronic SAC treatment can ameliorate cognitive deficits in STZ-diabetic rats through modulation of nuclear factor (erythroid-derived 2)-like 2, nuclear factor-kappa B (NF-κB), toll-like receptor 4, heme oxygenase 1, and acetylcholinesterase and attenuation of associated oxidative stress and pro-inflammatory cytokines such as interleukin (IL)-1α, IL-1β and IL-6 (Baluchnejadmojarad, Kiasalari, Afshin-Majd, Ghasemi, & Roghani, 2017). SAC can also affect NF-κB activity and expression through modifying the mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathways. Through downregulation of the p38 and MAPK/ERK pathways, SAC inhibits NF-κB transactivation, mRNA expression and protein synthesis in diabetic kidneys (Colin-Gonzalez *et al.*, 2015).

The nephroprotective effect of TAU has been demonstrated by suppressing markers of oxidative stress, morphological and biochemical changes in kidneys of diabetic rats (Hsu & Tain, 2020; Koh *et al.*, 2014). Moreover, the mechanism of action of TAU in preventing kidney damage is attributed due to suppression of high glucose-induced signals activation, such as signal transducer activator of transcription-3 (STAT-3) and MAPK (Huang, Chuang, Guh, Huang, & Hsu, 2007). Furthermore, TAU modulates the cytochrome P450 2E1 activation that metabolizes a range of exogenous and endogenous substances and is an indicator of ROS in the kidneys of diabetic rats (Yao *et al.*, 2009). Therefore, TAU may prevent kidney damage in diabetic groups through the prevention of ROS generated by AGE and glucose in kidneys (Abdoli, Sadeghian, Azarpira, Ommati, & Heidari, 2021; Koh *et al.*, 2014; Winiarska, Szymanski, Gorniak, Dudziak, & Bryla, 2009). Also, TAU supplementation helped to prevent the onset and progression of DN by lowering blood glucose levels, upgrading glomerular basement membrane metabolism and lipid metabolism (Lin *et al.*, 2010).

Conclusions

In conclusion, the synergistic effect of SAC and TAU was comparable with that of glibenclamide against NA/STZ induced diabetes. Results of the present study prove the efficacy of combined action of SAC and TAU in lowering glucose levels and improving oxidative stress destruction in the kidneys of a medically relevant rat model of T2DM. Thus, SAC and TAU when given in combination may be regarded as a potential candidate and a viable option for managing diabetes-related complications. However, further research and developmental work is required for this combination to be considered an additional and as an alternative therapeutic drug for the treatment of diabetes and a prophylactic measure as well.

Limitations of the study

It is imperative to perceive some limitations of this study. The results of our research need to be confirmed with additional focus on other animal species and human studies. Moreover, the fundamental ideas of this investigation opened up a number of new frontiers for future

researchers to pursue in order to conduct similarly extensive studies that establish novel pharmacological aspects in order to clarify the synergistic effects of various dosage forms with regard to their mechanisms of action.

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