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Research Article

Canagliflozin exerts anti-inflammatory and antioxidant effects in the heart and skin tissues: Biochemical and histopathological assessment in a model of accelerated aging induced by D-galactose in mice

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Abstract

Canagliflozin was assessed for its anti-inflammatory and antioxidant effects as an anti-aging drug in animal models. 50 Swiss albino male mice were divided into five groups; all groups received their intervention using gastric gavage; group 1 received normal saline for 14 weeks; group 2 received induction by D-galactose 200 mg/kg/day for seven weeks; and group 3 to 5 received the same induction for seven weeks, followed by another seven weeks of investigated drugs; group 3 received Vitamin C (100 mg/kg/day); group 4 received canagliflozin (3 mg/kg/day); and group 5 received canagliflozin (1 mg/kg/day). At the end of 14 weeks, all animals were euthanasiad, and heart and skin tissue were harvested for further analysis. Canagliflozin at both 1 and 3 mg/kg was successful in reducing the levels of inflammatory mediators (TNF-alpha and IL6), reducing levels of MDA, increasing the levels of SOD, and increasing the levels of collagen-1 and elastin in skin tissue. Additionally, 3 mg/kg Canagliflozin showed a better effect compared to 1 mg/kg regarding its effect on IL6, SOD, and elastin. Histopathologically, treatment with both doses of Canagliflozin attenuates abnormalities induced by D-galactose (bizarre, irregular, and hyperchromatic nuclei). Canagliflozin exhibits potent antioxidant and anti-inflammatory effects in living organisms, effectively prevents cardiac and skin damage generated by D-galactose, and possibly reduces aging.

Keywords

canagliflozin, inflammation, oxidative stress, mice, aging, d-galactose

Introduction

Aging refers to the progressive decline of the physiological functions essential for survival and reproductive capability over time. Natural aging differs from age-related disorders like cancer and heart disease (Xing et al. 2023). Understanding that the aging process should not be categorized as a disorder is crucial since it involves all organs and tissues. Simultaneously, illnesses usually display more localized symptoms (Bulterijs et al. 2015).

The aging process is marked by widespread and long-term inflammation (Ferrucci and Fabbri 2018). The deterioration of cells triggers this inflammation, weakened immune system function, impaired organ performance, and the development of age-related illnesses (Ponnappan and Ponnappan 2011). Due to the intricate nature of aging, it is crucial to organize inflame-aging systematically by reducing its components. The senescence-associated secretory phenotype (SASP), which comprises factors released by senescent cells, promotes chronic inflammation and can trigger senescence in healthy cells. Simultaneously, persistent inflammation speeds up the aging process of immune cells, leading to a compromised immune system and an incapacity to eliminate aging cells and inflammatory substances; this establishes a harmful loop of inflammation and aging (Li et al. 2023). Thus, inflammation has been acknowledged as an intrinsic part of aging, and its disappearance could be a viable anti-aging approach.

Many internal and external mechanisms generate reactive oxygen and nitrogen species (RONS), and antioxidant defenses counteract their detrimental impacts; the oxidative stress theory of aging states that age-related declines in function result from the buildup of damages caused by RONS (Liguori et al. 2018). The precise mechanism by which oxidative stress induces aging remains unclear, although it is likely that elevated levels of RONS contribute to cellular senescence. Senescent cells develop a permanent senescence-associated secretory phenotype (SASP) characterized by the release of soluble substances (such as interleukins, chemokines, and growth factors), degradative enzymes such as matrix metalloproteases (MMPs), and insoluble proteins/extracellular matrix (ECM) components (Pole et al. 2016; Chandrasekaran et al. 2017).

An innovative and promising strategy involves repurposing clinically approved medications easily accessible as dietary supplements. Several type 2 diabetes medications have become more popular for their ability to postpone the aging process by regulating glucose metabolism and insulin action (Kalyani and Egan 2013). A recent study demonstrated that canagliflozin (CAG), a type 2 sodium-glucose cotransporter inhibitor, effectively slowed the development of age-related abnormalities in male mice (Snyder et al. 2023). Recent research has demonstrated that CAG, a safe and efficient medicine for treating type 2 diabetes, can slow down age-related damage in male mice. However, its effectiveness in reducing such damage in female mice is comparatively lower (Wezeman and Ladiges 2022). Some have suggested that CAG exerts possible anti-aging activity via AMPK activation since it can inhibit mTOR to promote lifespan (Thanapairoje et al. 2023).

Despite these promising results in male mice, the exact molecular mechanism of CAG as an anti-aging drug is unclear. We undertook this study to shed light on some of the molecular mechanisms of aging, specifically the inflammatory and oxidative stress mechanisms in heart tissue and changes in elastin and collagen in skin tissue. The study aims to examine the effect of CAG as an anti-aging drug in animal models.

Methods

Study design and setting

A group of male Swiss albino mice were selected for the study. The mice had a mean body weight range of 20–40 g and an age range of 3–7 months. They were randomly divided into five groups, each containing 10 mice housed in separate cages. In total, 50 mice were used in the study, as shown in Table 1. The rodent was housed in a polypropylene enclosure within a regulated environment, maintaining an ambient temperature of 23 ± 4 °C. The lighting settings were adjusted to a standard 12/12-hour light-dark cycle. Before the start of the investigation, the mice were acclimated for 14 days at the Animal Facility of Al-Mustafa University College in Baghdad, Iraq. The rodents were supplied with a consistent pellet diet and unlimited access to water. The study was prepared following the ARRIVE guidelines 2.0.

The animal was allocated using block design (see Table 1), and all oral drugs were administered using gastric gavage. Canagliflozin (INVOKANA®, Janssen Pharmaceuticals, Inc., USA) was formulated as a suspension in 0.5% hypromellose (Mamidi et al. 2014).

Induction of aging was done using D-galactose (Sigma Aldrich®, USA) at 200 mg/kg/day for seven weeks (Chogtu et al. 2018; Martinovic et al. 2023; Obaid and Fawzi 2024). A successful induction is distinguished by disheveled fur and a generally fuller physical appearance. Moreover, older mice may display diminished vigilance, decreased physical activity levels, wrinkled skin, reduced responsiveness, or increased hesitation compared to younger mice (Toth 2018).

Laboratory analysis

Following the completion of the treatment intervention, euthanasia was performed on every animal after 14 weeks. All of them completed a 10-hour fast. Afterwards, the subjects received intraperitoneal (IP) anesthesia with a dose of 80 mg/kg of ketamine and 10 mg/kg of xylazine. Following the administration of full anesthesia, the mice were euthanized employing carbon dioxide (Underwood and Anthony 2020; Yaribeygi et al. 2023). Subsequently, a postmortem dissection was performed on the dead animals. This dissection aimed to remove the heart and skin (Chen et al. 2021; Khafaji et al. 2024; Maded et al. 2024).

Table 1. Study groups and their administered drugs.

	Induction [a]	Intervention	Time
G1 (Obaid 2024)	-ve	Normal saline	14 weeks
G2 (Chogtu et al. 2018; Martinovic et al. 2023; Obaid and Fawzi 2024)	+ve	Normal saline	14 weeks
G3 (Li et al. 2019; Obaid and Fawzi 2024)	+ve	Vitamin C (100 mg/kg/day)	14 weeks
G4 (Mamidi et al. 2014)	+ve	Canagliflozin (3 mg/kg/day)	14 weeks
G5 (Liang et al. 2012)	+ve	Canagliflozin (1 mg/kg/day)	14 weeks

[a] Induction was done for 7 weeks at a dose of 200 mg per kg daily using d-galactose orally.

After applying phosphate-buffered saline (PBS) with a pH of 7.4, histological investigation was performed on heart tissue. Subsequently, the conventional processing methodology utilizes the paraffin-embedded technique (Sadeghipour and Babaheidarian 2019).

The collected tissue was pulverized using an electric tissue homogenizer device (Staruar®, England). The homogenate was centrifuged using a Thermos Scientific® centrifuge from the USA at a temperature of four degrees Celsius and a speed of 2000 revolutions per minute for 20 minutes. The aqueous fraction was collected using a micropipette and stored at -20 °C until the analysis day.

In the ELISA procedure, 50 milligrams of tissue were placed in an Eppendorf tube from Eppendorf®, Germany, with 0.45 milliliters of cold PBS. Afterward, the tissue was carefully cut into extremely little pieces.

Biochemical analysis

A double-sandwich ELISA method (Cortez Diagnostics®, USA) was used for biochemical testing. ELISA is used to measure the levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), glutathione peroxidase (SOD), malondialdehyde (MDA), collagen I (Col-I), and elastin (ELN) using specific ELISA kits (MyBioSource, USA).

Histopathology assessment

The histopathologist analyzed cardiac tissue using the H&E stain and observed the structure of the heart's myocytes using a light microscope (Olympus BX51 Microscope, Olympus Corporation®, Japan). Random inspections were conducted in five regions of a slide corner and the middle area, using a magnification power of X40.

Ethical approval

The study was approved by the research ethical committee of the Al-Mustafa University College (ID: AP018, date: 11 November 2023).

Statistical analysis

All analyses were carried out by MedCalc version 14 (Ostend, Belgium). The continuous variables were compared using ANOVA analysis, and each pair was compared using the post hoc Tukey test. The level of significance was 0.05.

Results

Inflammatory mediators in heart tissue

Mice induced by D-galactose (G2) showed significantly higher levels of inflammatory mediators (TNF-alpha and IL-6) than the normal control group (G1), indicating successful induction. In mice treated with CAG at 1 and 3 mg/kg per day and vitamin C (positive control), inflammatory mediators' levels were statistically low compared to the induction group. Furthermore, the levels of IL-6 were significantly lower in mice treated with CAG at a dose of 3 mg/kg compared to those treated with 1 mg/kg, as illustrated by Fig. 1.

Oxidative stress markers in heart tissue

Mice induced by D-galactose showed significantly higher levels of MDA than the normal control group, indicating successful induction. In mice treated with CAG at 1 and 3 mg/kg per day and vitamin C (positive control), the MDA levels were statistically low compared to the induction group, as illustrated by Fig. 2A.

In mice treated with CAG at both 1 and 3 mg/kg per day and vitamin C (positive control), the levels of SOD were significantly higher compared to the induction group. The levels of SOD were significantly higher in mice treated with CAG at a dose of 3 mg/kg compared to those treated with 1 mg/kg, as illustrated by Fig. 2B.

Skin markers (collagen and elastin)

Mice induced by D-galactose showed significantly lower levels of collagen-1 and elastin than the normal control group, indicating successful induction. In mice treated with CAG at both 1 and 3 mg/kg per day and vitamin C (positive control), the levels of collagen-1 and elastin were statistically higher compared to the induction group. Furthermore, elastin levels were significantly higher in mice treated with CAG at a dose of 3 mg/kg than those treated with 1 mg/kg, as illustrated by Fig. 3.

Histopathology of heart tissue

Fig. 4A depicts normal cardiac cells forming a syncytium of cardiac fibers with central nuclei. Some fibers possess intercalated discs that exhibit a pale pink hue. Red blood cells are observed to be aligned linearly within capillaries among the fibers. Fig. 4B depicts the induction group

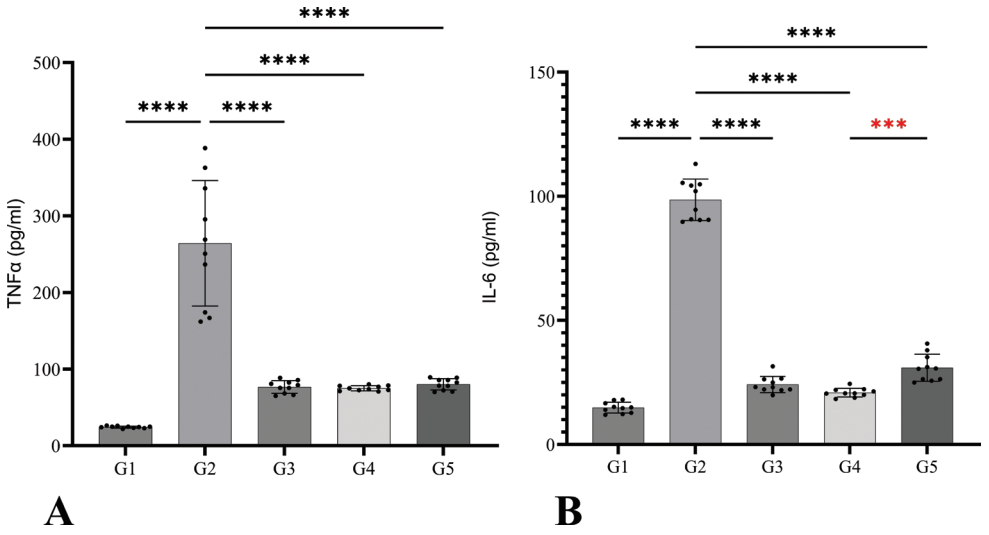

Figure 1. Assessment of inflammatory markers in cardiac tissue **A.** TNF-alpha; **B.** IL-6. One-way ANOVA with *post hoc* Tukey test, *** indicate p-value ≤ 0.001, and **** indicate p-value ≤ 0.0001.

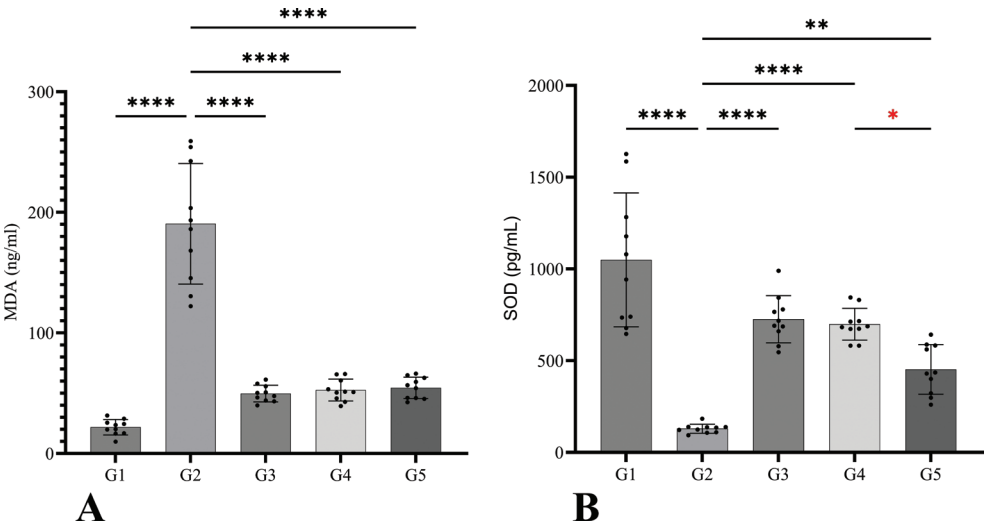

Figure 2. Assessment of oxidative stress markers in cardiac tissue. **A.** MDA levels; **B.** SOD levels. One-way ANOVA with *post hoc* Tukey test, * indicates p-value ≤ 0.05, ** indicate p-value ≤ 0.01, and **** indicate p-value ≤ 0.0001.

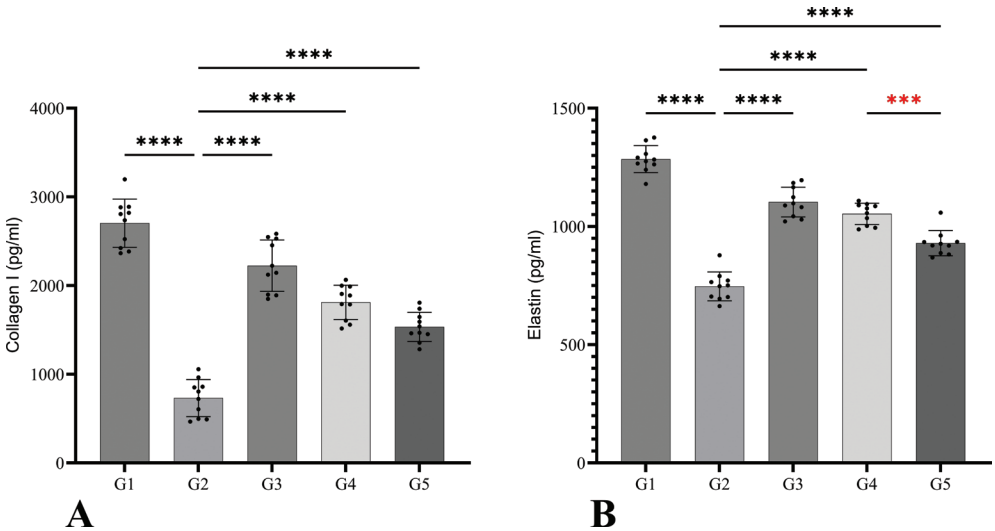

Figure 3. Assessment of skin markers. **A.** Collagen 1; **B.** Elastin. One-way ANOVA with *post hoc* Tukey test, *** indicate p-value ≤ 0.001, and **** indicate p-value ≤ 0.0001.

exhibiting abnormal, irregular, and densely stained nuclei. Fig. 4C illustrates the cardiac tissue of an animal administering 1 mg/kg CAG, and Fig. 4D shows the cardiac tissue of an animal administering 3 mg/kg CAG. It reveals the presence of irregular and hyperchromic nuclei, indicating the beginning of the healing process in the heart tissue toward normal cells, with 3 mg/kg animals showing better healing.

Discussion

Our study showed for the first time that CAG protects against D-galactose-induced oxidative and inflammatory damage in mice's heart and skin tissue, possibly indicating its anti-aging potential. CAG was successful in reducing the levels of oxidative stress and inflammatory markers and improving skin elastin and collagen levels.

At the biological level, the process of aging is attributed to the effects of the gradual accumulation of diverse forms of molecular and cellular damage over an extended period. Consequently, there is a progressive decline in both physical and mental capabilities, an escalating susceptibility to illnesses, and, finally, mortality (Liguori et al. 2018). Approved anti-aging drugs target one or more molecules to reduce cellular damage and prolong the health span; these include the use of metformin (Ng et al. 2014; Lu et al. 2016; Stynen et al. 2018), rapamycin (Neff et al. 2013; Arriola Apelo and Lamming 2016; Carosi and Sargeant 2019), resveratrol (Park et al. 2012; Xia et al. 2017; Zhu et al. 2018), and Senolytics (Cox et al. 2015; Rossman et al. 2018; Hickson et al. 2019). Despite these efforts, anti-aging remains a very promising and yet challenging field. Because of the limited clinical efficacy and possible adverse drug reactions in humans and animals, many attempts are being made to find new therapies for anti-aging. To address the previous issues about anti-aging medications, this research examined the effect of CAG, which possesses antioxidant and anti-inflammatory properties in animal models, to shed light and promote future directions toward continuing the study of aging biology.

In the present study, D-Gal induced an aging process in mice; it increased the inflammatory mediator's levels in heart tissues, including TNFα and IL6, and also increased oxidative stress markers in the heart, including MDA, while decreasing antioxidant enzyme levels, including SOD; furthermore, it decreased COL-I and ELN levels in skin tissues and induced hypertrophy of cardiac cells. D-Gal-induced cardiac aging models have been shown in several investigations to result in increased ventricular

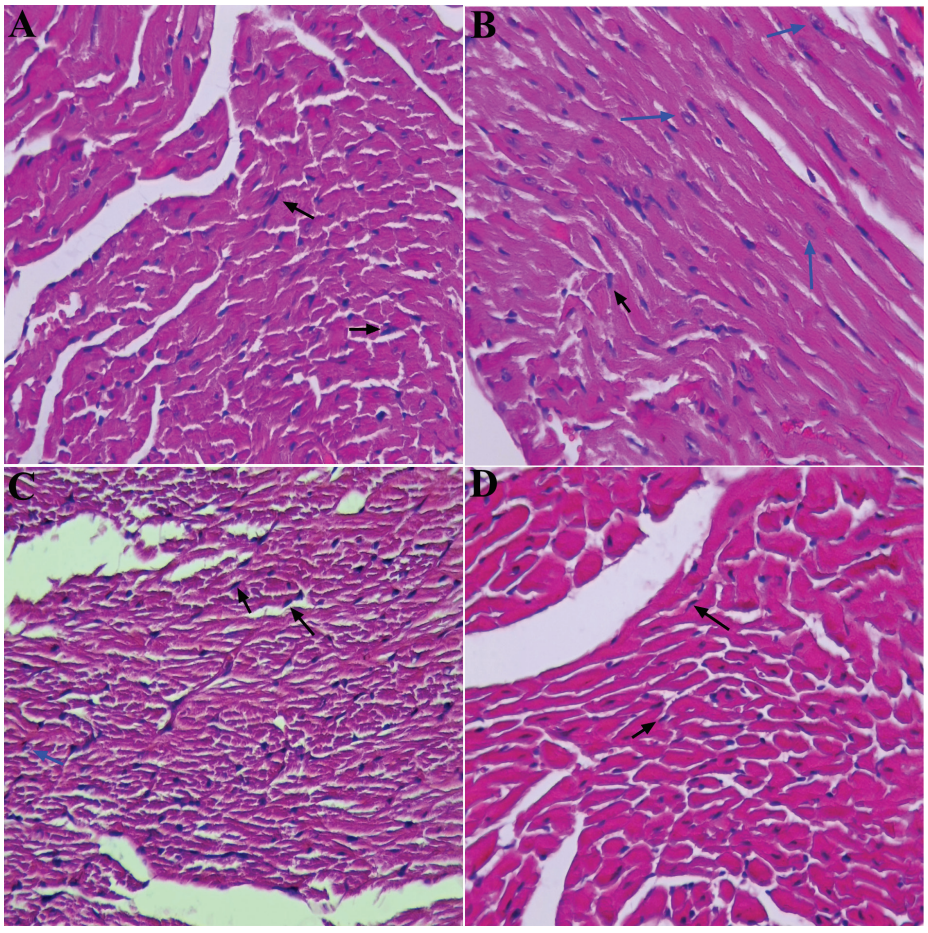

Figure 4. Light microscope histopathological images of hematoxylin- and eosin-stained cardiac tissue. **A.** G1: no treatment; **B.** G2: induction group; **C.** G5: 1 mg/kg canagliflozin, and **D.** G4: 3 mg/kg canagliflozin. The black arrow indicates normal cardiac cells and normal nuclei, and the blue arrow indicates bizarre, irregular, and hyperchromatic nuclei. X40.

hypertrophy and cardiac inflammatory cells (Cebe et al. 2014; Chang et al. 2016; Chang et al. 2017; Liang et al. 2017). D-Gal may contribute to heart hypertrophy by increasing oxidative stress due to metabolism disruption, including galactose oxidation and developing advanced glycation end products (AGE). Also, AGE-RAGE (RAGE: receptor of AGE) interactions in cardiac tissue can activate nuclear factor kappa B (NF-κB) translocation and boost inflammatory gene transcription, leading to inflammation (Frimat et al. 2017; Hussein et al. 2024).

In the present study, levels of TNFα and IL6 in heart tissue were significantly reduced in animals given CAG at both doses compared to the induction group; additionally, it appears that 3 mg/kg CAG showed a more beneficial reduction of IL6 level compared to 1 mg/kg. These outcomes prove that SGLT2 inhibitors may have broader antioxidant and anti-inflammatory effects across the body and specific effects on heart tissue. The main mechanism by which canagliflozin acts as an antidiabetic is by inhibiting glucose reabsorption through the blockage of SGLT2. This glucose transporter is predominantly found in the kidneys and intestines but not in other tissues like the heart (Chen et al. 2010; Sayour et al. 2019). These effects are not influenced by the unique expression of SGLT2 in organs and are likely accompanied by numerous implications (Chen et al. 2010; Sayour et al. 2019).

Hasan et al. investigated the protective effect of CAG in heart tissue against oxidative stress induced by isoprenaline in Long-Evans rat hearts; CAG attenuates oxidative stress and apoptotic processes, which was achieved by lowering the effect of nitric oxide synthase, transforming growth factor beta, and caspase-3, which are all involved in promoting oxidative stress, inflammation, and cell death (Hasan et al. 2020).

One of the most significant alterations with age is a dysregulation of the immune response that results in a chronic systemic state of inflammation. Cytokines and chemokines are two examples of dysregulated proinflammatory mediators that play a significant role in the onset of chronic inflammation and immunosenescence (Chung et al. 2019).

In the present study, the levels of MDA and SOD were improved with treatment with CAG at both doses; moreover, 3 mg/kg showed better results for SOD compared to 1 mg/kg. SGLT2 inhibitors function as indirect antioxidants by reducing oxidative damage caused by elevated glucose levels. Furthermore, studies have demonstrated that SGLT2 inhibitors effectively decrease the production of free radicals (Steven et al. 2017), inhibit pro-oxidants such as NADPH oxidase 4 (Tahara et al. 2014; Terami et al. 2014), and increase the activity of antioxidant enzymes such as SOD (Osorio et al. 2012; Oshima et al. 2019; Yaribeygi et al. 2019). While most of these studies examined empagliflozin or dapagliflozin, the current study's uniqueness is that it examined the antioxidant effect of CAG for the first time. It revealed that it has potent antioxidative effects in a dose-dependent manner.

Our research indicates that canagliflozin is involved in numerous pathways for antioxidant and anti-inflammatory effects. Therefore, it is possible that other molecular processes, apart from the ones mentioned above, contribute to the cardioprotective effects of canagliflozin. Due to the consistent connection between diabetes and oxidative stress and inflammation in the cardiovascular system, we utilized a model of induced cardiac oxidative stress and aging to examine the antioxidant capabilities of canagliflozin. This approach allowed us to focus solely on the effects of canagliflozin without any interference from other diabetes-related mechanisms. Hence, further investigations using diabetic animal models are necessary to fully comprehend the therapeutic capabilities of this medication concerning oxidative stress and organ damage caused by D-galactose.

Conclusion

Our research revealed that canagliflozin exhibits potent antioxidant and anti-inflammatory effects in living organisms, effectively prevents cardiac and skin damage generated by D-galactose, and possibly reduces aging. These actions may entail various pathways beyond localized actions in the heart and have broad systemic effects.

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