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Original article

Effect of Ashwagandha (*Withania somnifera*) on cardiac aquaporins in adolescent rats under a chronic unpredictable mild stress model

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Abstract

Adolescent depression is a prevalent and serious mental health issue that can negatively impact multiple organ systems, including the cardiovascular system. This study investigates the effects of chronic unpredictable mild stress (CUMS), a validated animal model of depression, on the expression of Aquaporins (AQP1, AQP2 and AQP3) in the cardiac tissue of adolescent rats and evaluates the potential therapeutic role of ashwagandha (*Withania somnifera* (L.) Dunal). Twenty-eight male Wistar albino rats were divided into four groups: Control, CUMS, CUMS+SERT, and CUMS+ASHW. Treatments were administered via oral gavage for 28 days, and cardiac tissues were evaluated histologically, immunohistochemically and biochemically. While serum levels of CK, Na, Cl and K showed no significant differences across groups, histological examination revealed myocardial damage (edema, hemorrhage, vacuolization) in CUMS rats. Immunohistochemical analysis demonstrated strong AQP1 expression across all groups, while AQP2 and AQP3 showed increased expression in treatment groups compared to the control. These findings suggest that Ashwagandha may mitigate stress-induced cardiac changes and influence aquaporin expression, particularly AQP2 and AQP3, which could be relevant to cardiac water balance and function in depression. Further studies are warranted to explore the molecular mechanisms linking aquaporin modulation and cardioprotection in stress-related pathologies.

Keywords: ashwagandha, aquaporins, depression, rat, sertraline

Introduction

Depression is a multifactorial mental disorder that has been known since ancient times and remains a significant public health problem. Adolescent depression shares many clinical features with adult depression and is quite common during this developmental period (Georgotas 1988). In adolescents, symptoms such as social withdrawal, anhedonia and suicidal ideation can significantly impair psychosocial functioning (Cuijpers et al. 2023). In addition to its psychological burden, depression can also have negative effects on cardiovascular health. Chronic psychosocial stress activates neuroendocrine pathways, including the hypothalamus -pituitary-adrenal axis and the sympathetic nervous system; this can lead to endothelial dysfunction, inflammation, oxidative stress and autonomic imbalance, resulting in myocardial damage and cardiac dysfunction (Vaccarino 2024). Adolescence is a critical developmental period due to rapid neurobiological maturation, hormonal changes, and increased stress sensitivity. Stress exposure during this period can lead to long-term cardiovascular fragility through the developmental programming of stress response systems; therefore, investigating the effects of depression on the heart during adolescence may reveal early mechanisms preceding adult cardiovascular disease (Steptoe and Kivimäki 2013).

Creatine kinase (CK) is a well-known biomarker of myocardial cell damage, while serum electrolytes such as sodium, potassium and chloride are indicators of osmotic balance and cellular ion homeostasis. Previous studies have demonstrated significant associations between serum electrolyte levels (including sodium, potassium and chloride) and cardiac injury markers such as creatine kinase (CK) and CK-MB, suggesting a close interaction between electrolyte homeostasis and myocardial damage processes (Elhabiby et al. 2023).

Aquaporins (AQPs) are proteins that regulate the passage of water across the cell membrane and were named „aquaporin" in 1993 (Benga 2006). AQPs found in tissues such as the kidney, brain and eye, may also play a protective role in stress conditions, such as dehydration and ischemia, by being expressed in the heart muscle. Cardiac water balance is important for myocardial contractility; excessive fluid accumulation may lead to loss of function (Kuriyama et al. 1997, Mehlhorn et al. 2001). Disruptions in cardiac water balance can lead to myocardial edema and functional disorders (Shangzu et al. 2022, Tüfekçioğlu et al. 2023). AQPs isoforms exhibit a differential distribution in cardiac tissue. AQP1 is the best-defined water channel in the heart and is predominantly found in endothelial cells and cardiomyocytes. In contrast, the roles of AQP2 and AQP3 in the cardiovascular system are less characterized. Current data suggest that AQP2 may exert indirect cardiac effects via vasopressin-mediated fluid balance pathways, while AQP3 may contribute to water and glycerol transport, oxidative stress, and inflammatory processes (Shangzu et al. 2022). Furthermore, the involvement of AQPs in processes such as cell volume regulation, cell migration, and inflammation indicates that these channels are active regulators in cardiovascular pathophysiology (Shangzu et al. 2022). Therefore, the inclusion of AQP2 and AQP3 in this study aimed to investigate their potential roles in cardiac tissue under chronic stress and to determine the possible effects of these less characterized aquaporins on myocardial water homeostasis and stress-induced cardiac changes. Sertraline was included in the study as a reference pharmacological treatment because it is a selective serotonin reuptake inhibitor (SSRI) commonly used in the clinical management of adolescent depression. In addition to its antidepressant effects on the central nervous system, sertraline has been reported to affect neuroendocrine stress responses, oxidative balance and inflammatory pathways. Including sertraline as a comparison group allowed us to evaluate whether the potential cardioprotective and aquaporin -modulating effects of ashwagandha were comparable to those of a standard antidepressant treatment (Gokdemir et al. 2025). However, ashwagandha, a medicinal herb widely used in traditional Indian medicine, has been reported to exhibit well-documented anti-stress and antidepressant-like properties in experimental and clinical settings, as well as immunomodulatory, anti-inflammatory and neuroplasticity-promoting effects (Kim 2025). The biological activity of ashwagandha is reported to be primarily due to its bioactive components, including withanolides, cytoindocides and alkaloids. These compounds have been shown to reduce oxidative stress, suppress pro-inflammatory cytokines, regulate cortisol levels, and improve mitochondrial function (Gökdemir et al. 2025). Through these antioxidant and membrane-stabilizing mechanisms, ashwagandha may indirectly modulate membrane permeability and cellular water homeostasis, potentially influencing aquaporin-related pathways (Singh et al. 2011, Das et al. 2025). Recent evidence indicates that antioxidant and anti-inflammatory phytochemicals may modulate aquaporin expression through the regulation of oxidative/osmotic stress signaling pathways and membrane channel trafficking mechanisms; however, the effects of ashwagandha on cardiac aquaporins under chronic stress conditions are largely unknown (Markou 2022). The presence of AQP channels has been detected in rat myocardium. Studies have reported that AQP channels play a role in myocardial

edema occurring in doxorubicin-induced myocardial infarction and cisplatin-induced myocardial injury. However, the function of aquaporin (AQP) channels in adolescent depression has not been fully elucidated (Tüfekçioğlu et al. 2023).

Ashwagandha is a herb widely used in traditional medicine and known to have anti-stress and anti-depressant properties. Extracts from studies on animals and clinical trials have revealed that *W. somnifera* roots and leaves possess significant anti-stress activity (Kim 2025). Research investigating the impact of ashwagandha and its active constituents on aquaporin expression in cardiac tissue during adolescent depression remains limited.

The protective effects of ashwagandha on stress and depression are well-established, while the association between myocardial edema, AQP channels, and ashwagandha remains to be well-demonstrated, Furthermore, while the expression and roles of AQP1 in the heart are well known in the literature, limited information is available regarding AQP2 and AQP3. Therefore, this study aimed to evaluate the effects of chronic unpredictable mild stress on AQP1, AQP2 and AQP3 expression in the cardiac tissue of adolescent rats and to investigate the potential therapeutic effects of ashwagandha in comparison with sertraline.

Materials and Methods

Animal supply and care conditions

Experimental protocols were approved by the Dicle University Prof. Dr Sabahattin Payzın Health Sciences Application and Research Center, Animal Experiments Local Ethics Committee (meeting number: 2-06/09, date of approval: February 29, 2024). The experiments were performed on 28 male Wistar Albino rats obtained from the Medical Science Application and Research Center of Dicle University. All animals (35-49 days old of age and weighing 150-180 g) were kept in a standard controlled environment (22 ± 1°C, 40–70 % humidity, 12:12 h light: dark cycle, ventilation 10 changes/hour, sound level up to 75 dB, day time light intensity up to 200 lux) and given rat chow and water ad libitum. The sample size was determined by an a priori power analysis performed using G*Power (version 3.1.9.7, Heinrich Heine University Düsseldorf, Germany). Based on previous studies using similar CUMS models, a moderate-to-large effect size (f=0.40) was assumed. With an alpha level of 0.05 and a desired statistical power (1−β) of 0.80 for one-way ANOVA comparisons among four groups, the minimum required sample size was calculated as 6 animals per group. To compensate for potential data loss and to ensure adequate statistical robustness, 7 animals were included in each group. This approach is consistent with previous experimental studies and complies with the 3R principles (Replacement, Reduction, and Refinement) for the ethical use of animals in research. (Gokdemir et al. 2025).

Rat age selection

In rats, the juvenile period, between 20 and 21 days, is characterized by increasing sociability, and the development of control mechanisms occurs between 25 and 35 days. Adolescence typically spans 35 to 49 days and is characterized by an increase in behavioral and cognitive control capacity (Semple et al. 2013). Therefore, 35- to 49-day-old rats were preferred for modeling the adolescent period in this study. This developmental period is considered similar to human adolescence in terms of neuroendocrine and behavioral maturation.

Chronic unpredictable mild stress model

The Chronic Unpredictable Mild Stress (CUMS) model aims to mimic depression by administering various unpredictable mild stressors to rats. In this model, rats are exposed to a variety of stressors for at least two weeks, including disruption of the light-dark cycle, continuous lighting, changing housing conditions, wet cage floors, dirty cages, deprivation of feed and water, and noise (Porsolt et al. 1978, Santiago et al. 2015).

For the CUMS model, 333 g of sawdust in the cage was wetted with 1.5 liters of water for wet cage application. For the tilted cage procedure, the cages were angled at a 60-degree angle so that the food part of the cage remained above. For 10 minutes, soiled and wet cat litter was left next to the cage. For the fasting stress, the feeds were collected from the cages at 16:00 and put back into the cages at 09:00 the next day (thus creating a 17-hour fasting period). Restraint stress was performed by physical restraint in the restraint apparatus (Santiago et al. 2015) .

The cages of rats in the control group and the cages of rats in the CUMS model were kept in separate rooms. The animals were not disturbed except for essential routine procedures, including cage cleaning. The stressors were applied in a randomized and unpredictable sequence to prevent habituation.

Experimental protocol

The rats were randomly divided into four groups (n = 7 per group):

1. Control
2. CUMS
3. CUMS+SERT
4. CUMS+ASHW

The control group animals were maintained under standard conditions and received 0.5 cc of physiological saline by oral gavage. Rats in the CUMS group were exposed to the stress protocol and also received 0.5 cc of physiological saline by oral gavage.

Treatment protocols were applied to rats in the CUMS+SERT and CUMS+ASHW groups starting from the 3rd day of CUMS exposure.

Sertraline (Sertraline; Selectra 25 mg film-coated tablet) was administered via oral gavage at a dose of 5 mg/kg/day (0.5 cc), dissolved in water, 1 hour before exposure to stressors (Pereira-Figueiredo et al. 2014).

Ashwagandha (Withania somnifera extract, Sigma-Aldrich Chemie GmbH) was administered via oral gavage at a dose of 50 mg/kg/day (0.5 cc), 1 hour before exposure to stressors (Bhattacharya et al. 2001, Gökdemir et al. 2014).

Treatments were continued daily from day 3 to day 17.

Tissue collection

Euthanasia was performed by cardiac puncture under anesthesia with xylazine (10 mg/kg) and ketamine HCl (90 mg/kg), administered intraperitoneally. Blood was withdrawn from the left ventricle, and cardiac tissue samples were collected for histological and immunohistochemical analyses.

Histomorphological and immunohistochemical analysis

Left ventricular tissue samples were fixed in 10% neutral buffered formalin for 48 hours. After fixation, all tissues were washed under tap water, dehydrated through an ascending graded ethanol series, and embedded in paraffin.

Histopathological evaluation

Serial sections of 5 μm thickness were taken from paraffin-embedded cardiac tissue blocks taken from sacrificed animals. To show histopathological changes, sections were stained with Crossman's triple stain and viewed under a light (NIS Elements Imaging Software (version 3.10) (Bancroft and Cook 1984).

For the histopathological evaluation, the sections were subsequently examined under a light microscope for hemorrhage, congestion, intracellular and extracellular edema, necrosis and myocardial degenerative changes. Degenerative changes in the myocardium were considered in the presence of striation loss, vacuolar changes and membrane irregularities. The histopathological damage score was calculated according to the severity of tissue damage as follows: 0 (none), 1 (mild), 2 (moderate), and 3 (severe) (Türkmen et al. 2022).

Immunohistochemical evaluation

Serial sections of 5 μm thickness were taken from paraffin-embedded cardiac tissue blocks taken from sacrificed animals. Streptavidin-biotin peroxidase immunohistochemical staining method was applied to the sections to determine aquaporins (AQP1, AQP2, AQP3). After deparaffinization and rehydration processes, paraffin sections were washed with distilled water. The sections were then first treated with 3% H_2O_2 in distilled water for 20 minutes and then washed three times in 0.01 M phosphate-buffered saline (PBS) for 5 minutes each to terminate endogenous peroxidase activity. To block nonspecific binding, sections were incubated in a blocking serum (His-tostain Plus Bulk Kit, Zymed) for 5 min at room temperature. Aquaporin antibodies (anti-aquaporin 1 Rabbit polyclonal, abcam, ab15080; anti-aquaporin 2, Rabbit polyclonal, abcam, ab15081; anti-aquaporin 3 Rabbit polyclonal, abcam, ab125219) were then added to the sections and left at room temperature overnight. The sections were then washed 3 times in 0.01 M PBS and incubated with biotinylated secondary antibodies (HistostainPlus Bulk Kit, Zymed) for 20 min in a humidified chamber at room temperature. After the sections were washed 3 times in PBS, they were treated with enzyme-conjugated streptavidin (Histostain Plus Bulk Kit, Zymed) for 20 minutes. The sections were washed again 3 times in PBS, and the reaction was visualized using 3,3'-diaminobenzidine tetrahydrochloride (Invitrogen, Carlsbad, CA). For counterstaining, sections were exposed to Gill hematoxylin for 1 minute and washed under running tap water until they turned blue. Sections were passed through alcohol, cleared in xylene, and coverslipped with Entellan. Stained preparations were viewed, and images were captured using a Nikon-Eclipse 400 DSRI microscope equipped with a Nikon digital camera and NIS Elements Imaging Software (version 3.10).

İmmunohistopathological evaluation. The results were evaluated semi-quantitatively. In the semi-quantitative evaluation, staining results were expressed as no staining (-), weak staining (+), moderate staining (++) and intense staining (+++) (Karakoç et al. 2016).

Biochemical analysis

Blood samples were collected into serum tubes (BD Vacutainer®) and centrifuged at 1500 g for 10 minutes. Serum levels of Creatine Kinase (CK), potassium (K), sodium (Na), and chloride (Cl) were measured using

Table 1. Values of biochemical parameters between rat groups.

Parameter	Control	CUMS	CUMS + SERT	CUMS + ASHW
Potassium (mmol/L)	6.0±1.47	5.37±1.37	6.71±1.20	7.26±1.50
Clor (mmol/L)	100.85±28.10	87.0±27.84	100.42±1.98	100.57±6.02
Chloride (U/L)	651.85±155.31	1059.42±480.45	1148.42±456.37	1231.28±743.64
Sodium (mmol/L)	137.71±36.52	121.0±38.65	138.71±2.36	137.57±8.50
P	NS	NS	NS	NS

NS: Non-significant

an automated biochemical analyzer (Architect c8000, Abbott). These parameters were selected as indicators of myocardial injury, electrolyte balance, and systemic metabolic alterations associated with chronic stress and treatment interventions.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 27.0. The Shapiro-Wilk test was applied to assess the normality of the data distribution. For data meeting the normality criteria, comparisons among the four experimental groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine pairwise group differences. In cases where the data did not follow a normal distribution, the Kruskal-Wallis H test was applied as a non-parametric alternative. All results were expressed as mean ± standard deviation (M ± SD), and statistical significance was set at a p-value of ≤ 0.05.

Results

Serum biochemical biomarkers

Serum biochemical parameters were assessed in all experimental groups, including potassium, chloride, creatine kinase (CK), and sodium levels (Table 1, Fig 1). No statistically significant differences were observed among the groups for any of the measured parameters ($p>0.05$).

Potassium levels were 6.0±1.47 mmol/L in the control group, 5.37±1.37 mmol/L in the CUMS group, 6.71±1.20 mmol/L in the CUMS+SERT group, and 7.26±1.50 mmol/L in the CUMS+ASHW group.

Chloride levels were 100.85±28.10 mmol/L in the control group, 87.0±27.84 mmol/L in the CUMS group, 100.42±1.98 mmol/L in the CUMS+sertraline group, and 100.57±6.02 mmol/L in the CUMS+ASHW group.

Creatine kinase (CK) activity was 651.85±155.31 U/L in the control group, 1059.42±480.45 U/L in the CUMS group, 1148.42±456.37 U/L in the CUMS+SERT group, and 1231.28±743.64 U/L in the CUMS+ASHW group.

Sodium levels were 137.71±36.52 mmol/L in the control group, 121.0±38.65 mmol/L in the CUMS group, 138.71±2.36 mmol/L in the CUMS+SERT group, and 137.57±8.50 mmol/L in the CUMS+ASHW group.

Histomorphological observations

Histological observations in all groups are shown in Fig. 2. The cardiac tissue of the control group was found to have a normal histological structure (Fig. 2A). Interstitial edema, hemorrhage foci, intracytoplasmic vacuolization, cardiomyocyte necrosis, and muscle fiber disarray were observed in the cardiac tissues of rats in the stress and treatment groups.

Interstitial edema and muscle fiber disarray persisted in the sertraline-treated group (Fig. 2B-C). Hemorrhage foci, edema, and necrosis were significantly reduced in the ashwagandha-treated group (Fig. 2D). The reduction in pathological changes in the treatment groups was also statistically significant (Table 2).

Immunohistochemical observations

AQP1, AQP2 and AQP3 immunohistochemical staining intensities in the heart are given in Table 3. AQP1 immunoreactivity was detected intensely in cardiac endothelial cells, cardiac myocytes and tubules, and blood vessel endothelial cells (Fig. 3 A-D). AQP3 immunoreactivity was found to be moderately intense, especially in cardiac myocytes and tubules. AQP2 immunoreactivity was observed to be weakly stained in endothelial cells and moderately stained in cardiac myocytes (Fig. 3E-H). In the comparison between the groups, it was determined that AQP1 immunoreactivity was intense in all groups, AQP2 immunoreactivity was weak in the control and treatment groups, and moderately stained in the stress group. AQP3 immunoreactivity was determined to be moderately stained in the control and stress groups, and weakly stained in the treatment groups (Fig. 3 I-L).

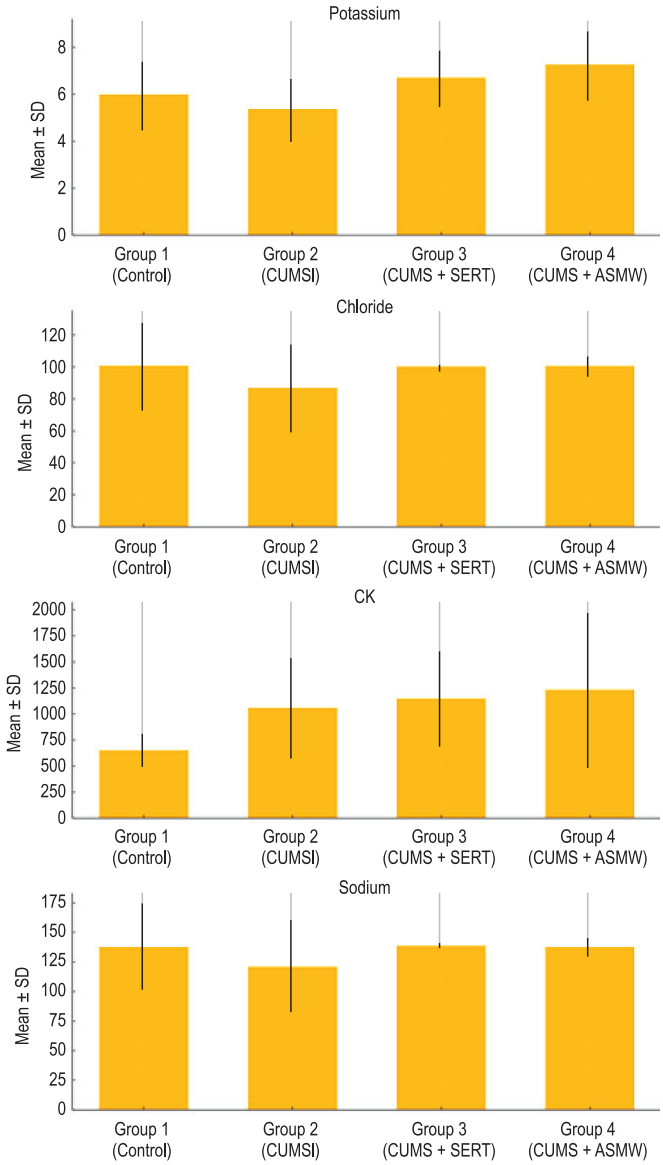

Fig. 1. Mean ± standard deviation values of biochemical parameters (Potassium, Chloride, CK, Sodium) across experimental rat groups. Although no statistically significant differences were detected ($p > 0.05$), numerical trends suggest potential physiological effects of CUMS and subsequent pharmacological (sertraline) and herbal (ashwagandha) interventions.

Discussion

Stress is a growing concern among adolescents. The process of adapting to new and challenging conditions can often trigger a pronounced stress response. The chronic unpredictable mild stress model is a frequently preferred method in experimental animal studies, especially those aimed at investigating the pathogenesis and management of depression. Earlier studies have demonstrated that various physiological changes occur in animals exposed to CUMS (Willner 2016). However, studies examining the effects of CUMS on cardiac structure and function are limited, and data on histopathological and immunohistochemical changes in the heart are particularly insufficient. Therefore, in the present study, the effect of stress exposure during adolescence on histopathological changes in myocardial tissue, AQP expression levels and selected some biochemical parameters was evaluated and the potential modulatory effects of ashwagandha, in comparison with sertraline, were also investigated.

Among the myocardial enzymes, CK is used as a primary clinical indicator to assess myocardial cell damage (Gökdemir et al. 2025). In the present study, although biochemical parameters did not show statistically significant differences, the numerical increase observed in CK levels in the stress and treatment groups may indicate early or subclinical myocardial stress that has not yet translated into systemic biomarker alterations. Similarly, mild fluctuations in sodium,

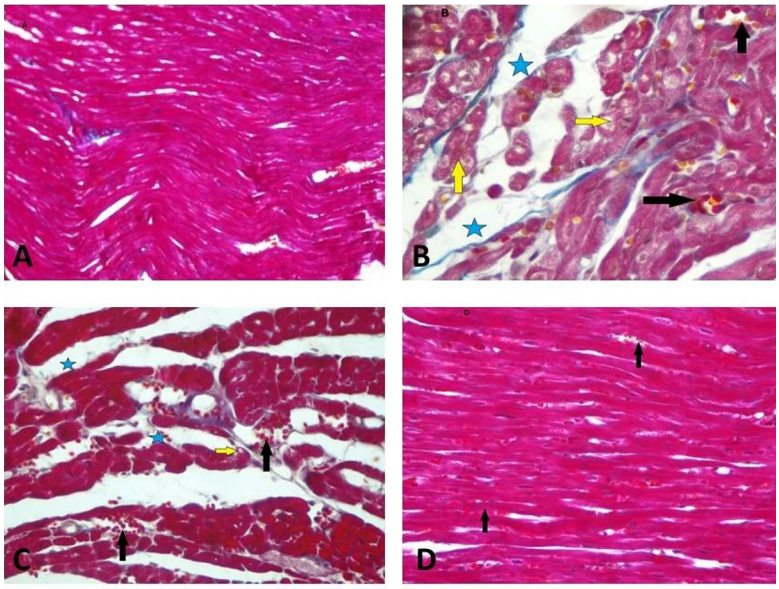

Fig. 2. Histomorphological appearance of each rat group. (A): Normal histological appearance in the control group heart, (B): Histopathological changes in the stress group, (C): Histopathological changes in the sertraline treatment group, (D): Histopathological changes in the ashwagandha treatment group. The cardiac tissue of the control group had a normal histological structure. Rats in the stress and treatment groups showed interstitial edema, hemorrhage foci, intracytoplasmic vacuolization, cardiomyocyte necrosis, and muscle fiber disorganization in their heart tissues. Interstitial edema and muscle fiber disorganization persisted in the sertraline-treated group. In the ashwagandha-treated group, hemorrhage foci, edema and necrosis were significantly reduced. Bleeding foci (black arrow), intracytoplasmic vacuolization (arrowhead), edema in the interstitium (asterisk), Staining: Crossman's Triple; Original magnifications A-D: x 20.

Table 2. Histopathologic scoring results obtained from rats.

Groups	Hemorrhage	Extracelluar edema	Necrosis	Degenerative changes
Control	0	0	0	0
CUMS	2 *	3 *	1	1
CUMS+ SERT	1 **	2 **	1	1
CUMS+ASHW	1***	1***	0	1

* p<0.001 CUMS group compared to control group. ** p<0.001 CUMS+SERT group compared to CUMS group. *** p<0.001 CUMS+ASHW group compared to CUMS group

potassium and chloride levels may reflect subtle changes in fluid-electrolyte balance, consistent with histological findings such as interstitial edema and alterations in aquaporin expression.

In line with this, interstitial edema, hemorrhage and myocardial degeneration were observed in the cardiac tissues of rats exposed to chronic stress. Sertraline, a commonly used antidepressant in the treatment of depression, obsessive-compulsive disorder, panic disorder, and anxiety, has long been considered to have a relatively safe cardiotoxicity profile; however, cases of cardiotoxic effects have been reported since the early 2000s (Ilgın et al. 2018). Although QT prolongation and adverse cardiac effects are rare, sertraline is generally regarded as safe and effective in the treatment of depression following myocardial infarction and is often recommended as a first-line SSRI in patients with cardiovascular disease (Gutlapalli et al. 2022). Experimental studies in rats have demonstrated that sertraline may induce cardiotoxic effects associated with increased serum CK levels, impaired myocardial performance, myofibrillar loss, vascular damage and interstitial edema (Ilgın et al. 2018). Consistent with these findings, the present study demonstrated pathological alterations in the sertraline group, including interstitial edema, hemorrhagic foci, intracytoplasmic vacuolization, cardiomyocyte necrosis, and disruption of muscle fiber organization, suggesting that sertraline may exert adverse cardiac effects under chronic stress conditions. These findings are consistent with previous reports showing that chronic stress causes endothelial dysfunction, increased vascular permeability and inflammatory responses in cardiac tissue (Ozmen et al. 2025). The lack of statistical significance in CK values, despite histopathological damage suggests that CUMS-induced myocardial changes in adolescent rats may occur at the

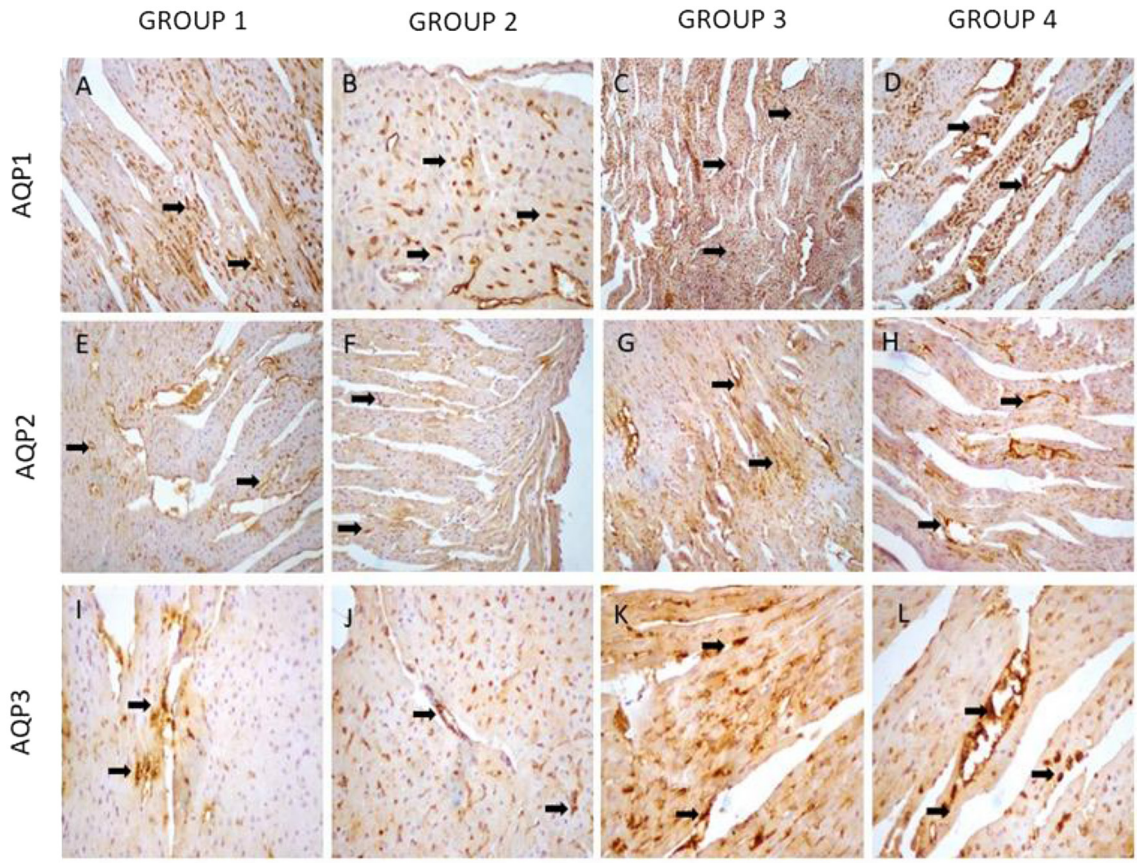

Fig. 3. AQP1, AQP2 and AQP3 immunohistochemical staining intensities in the heart. Black arrow: immunopositive reaction. AQP1 immunoreactivity was high in rat group, AQP2 immunoreactivity was moderate in the control, stress and treatment groups, while AQP3 immunoreactivity was moderate in the control and stress groups and weak in treatment groups. Original magnification A, C, E, F, G and H x 20, the others x 40.

Table 3. Immunohistochemical expression intensities of AQP1, AQP2 and AQP3 in the rat heart.

Groups	AQP1	AQP2	AQP3
Control	+++	+	++
CUMS	+++	++	++
CUMS+ SERT	+++	+	+
CUMS+ASHW	+++	+	+

Staining intensity: (-) no staining, (+) weak, (++) moderate, (+++) strong

tissue level before being reflected in circulating biomarkers. Although the biochemical findings in this study did not show statistically significant differences between groups, some numerical trends may still be biologically relevant. The increase in CK levels in the depression, sertraline, and ashwagandha groups may reflect stress-related myocardial tension or increased membrane permeability, consistent with histopathological evidence of myocardial changes. Similarly, slight fluctuations in sodium, potassium, and chloride levels may indicate subtle changes in fluid -electrolyte balance under chronic stress conditions. While these differences were not statistically significant, their direction is consistent with changes observed in aquaporin expression and myocardial water balance. These findings suggest that chronic stress can affect myocardial ionic and osmotic homeostasis even in the absence of apparent systemic biochemical disturbances. There are differing opinions in the literature regarding the effects of stress on CK activity (Pazini et al. 2019). While Segal et al. (2007) and Rai et al. (2003) reported increases in CK levels following stress, Reus et al. (2015) observed a decrease. Rezin et al. (2008), consistent with the present study, found no significant changes in CK levels. These differences can be attributed to various factors, including the duration and intensity of the stress model, the species of animal used, sex, age, and the type of biological material in which CK

levels were measured. Therefore, the biochemical findings may be interpreted as early-stage or subclinical cardiac stress rather than overt myocardial damage. Electrolyte findings also support this interpretation. While the decrease in sodium, potassium and chloride levels in the CUMS group may indicate mild disturbances in fluid-electrolyte balance under chronic stress, the return of these values to normal in the treatment groups suggests that systemic homeostasis has been partially restored. In particular, the increase in potassium levels in the group treated with ashwagandha may reflect adaptive cellular ionic responses related to membrane stability and cellular hydration status. Taken together, these biochemical findings are consistent with the histological observation of interstitial edema and support the hypothesis that stress alters myocardial water regulation.

Moreover, adolescence is a critical period characterized by accelerated neurobiological development, during which stress responses differ from those in adults (Romeo and McEwen 2006). These developmental characteristics may underlie the non-significant increase in CK levels observed in this study.

Cardiac function is dependent on water balance, and even a small increase in water content is known to impair contraction force (Rutkovskiy et al. 2013). Myocardial cells can regulate their volume by water transport across their plasma membrane, osmotic gradient and hydrostatic pressure. During extravasation, it has been reported that during extravasation water can flow between or across endothelial cells via diffusion, co-transporters such as ion channels, and aquaporin force (Wright and Rees 1998, Loo et al. 2002, Rutkovskiy et al. 2013). Recently, studies have been conducted on the potential roles of AQPs in cardiac pathophysiology. AQP1, AQP3, AQP4 and AQP7 are known to be expressed in cardiac tissue in humans and mammalian species (Adeoye et al. 2022, Roth et al. 2023). AQP1 and AQP4 are thought to play roles in cardiac edema and ischemia-reperfusion injury (Rutkovskiy et al. 2012, Ding et al. 2013). A stable osmotic environment is required for the normal transport of water and solutes in the body. Disruption of osmotic balance leads to pathophysiological conditions that affect many tissues and organs, including the heart, which can severely impair myocardial contractility and cardiac function. Studies have shown that aquaporins play a significant role not only in cardiac water homeostasis but also in cardiac excitation-contraction coupling by interacting with various ion channels and connexin proteins (Aggeli et al. 2021).

Fluid retention in cardiac tissue can occur in the interstitial space or manifest as myocardial edema; both are important because they cause cardiac dysfunction. The two main and most widely accepted factors underlying increases in interstitial fluid are increased myocardial filtration rates and decreases in myocardial lymph flow rates (Segal et al. 2007). The recent identification of novel AQP channels and their functions has led researchers to investigate the relationship between myocardial edema and AQP channels.

Aquaporin expression is tightly regulated by oxidative stress and inflammatory signaling pathways (Lv et al. 2023, Volkart et al. 2023, Cohen-Salmon et al. 2025). Considering the well-documented antioxidant and anti-inflammatory properties of ashwagandha, it is plausible that this phytotherapeutic agent may indirectly modulate AQP expression (Er et al. 2025, Gokdemir et al. 2025).

The main water channel identified in the hearts of mammalian species is known to be AQP1 (Benga 2006). Studies have reported that AQP1 is found not only in cardiac endothelial cells but also in human cardiac vascular smooth muscle cells and caveolae, and the T-tubules of rat cardiomyocytes (Tüfekçioğlu et al. 2023). AQP1 plays a central role in angiogenesis and cell migration in general, and although its molecular mechanism is not fully understood, it is known to involve AQP1-mediated water fluxes across the membranes of migrating cells. Cell migration is important for many biological processes, including angiogenesis, wound healing, tumor spreading and organ regeneration. AQP-dependent cell migration has been demonstrated in multiple cell types, including endothelial cells, astrocytes, epithelial cells (e.g., corneal and renal tubular cells), keratinocytes and tumor cells (Papadopoulos et al. 2008, Al-Samir et al. 2016). Among these, endothelial cells are particularly relevant for cardiac tissue, where AQP-mediated cell migration contributes to angiogenesis and microvascular remodeling. In a comparative study of cardiac AQPs in human, rat and mouse hearts, AQP1 expression was demonstrated in both cardiac myocytes and endothelial cells across species (Butler et al. 2006). In a study investigating the effect of hyperosmotic stress on aquaporins, it was reported that AQP1 membrane-associated proteins increased over time. High AQP1 levels directly increased water permeability and caused myocardial edema (Aggeli et al. 2021). Chronic stress and altered perfusion can disrupt myocardial fluid-electrolyte balance and create local osmotic gradients in cardiac tissue. Such microenvironmental changes are known to regulate AQP1 transport and abundance across the plasma membrane, thereby altering transmembrane water flow and contributing to myocardial water homeostasis (Rutkovskiy et al. 2013, Aggeli et al. 2021). Therefore, although our model does not directly trigger hyperosmotic stress, this paradigm suggests that stress

-related osmotic shifts may influence AQP1 behavior in cardiac tissue. These findings support the hypothesis that chronic stress may generate localized osmotic microenvironments in the myocardium that functionally resemble hyperosmotic conditions at the cellular level, thereby modulating AQP trafficking and contributing to myocardial water imbalance and interstitial edema. In this study, AQP1 immunoreactivity was intense in all groups, suggesting that its basal expression is preserved regardless of stress or treatment. This finding indicates that AQP1 may be constitutively expressed in cardiac tissue and less sensitive to mild chronic stress conditions, supporting previous experimental data (Rutkovskiy et al. 2013, Shangzu et al. 2022). However, these interpretations should be considered preliminary due to the limited sample size. While our findings are consistent with the notion that AQP1 is constitutively expressed in cardiac tissue and relatively stable under mild chronic stress, larger studies incorporating quantitative protein analyses and functional assays are required to confirm the magnitude and physiological significance of these observations.

In this study, AQP1 expression was found in endothelial cells and cardiac myocytes in all groups, which supports the literature. The similar immunreactivity intensities between the control and treatment groups suggest that depression treatment applied to rat cardiac tissue under mild stress has no notable effect on AQP1 expression.

AQP2 is a vasopressin-regulated water channel protein found in the connecting tubule and collecting duct, and is reported to play an important role in urine concentration and body water homeostasis (Kwon et al. 2013, Ozmen et al. 2025). AQP2 is a channel that is exclusively selective for water molecules and impermeable to ions or other small molecules. Although AQP2 has been reported to be absent from the cardiovascular system (Shangzu et al. 2022), recent studies have shown that AQP2 interacts in various tissues (Verkerk et al. 2019). Our study revealed that AQP2 is expressed in cardiac tissue, especially in cardiac myocytes and tubules. The fact that AQP2 immunoreactivity was weaker in the treatment groups compared to the control group suggests that AQP2 has a protective effect on edema in the cardiac tissue. The potential protective role of AQP2 in cardiac tissue may be related to its capacity to regulate transmembrane water flow and maintain intracellular-extracellular fluid balance. Under stress or impaired perfusion conditions, excessive water influx can contribute to cellular swelling and myocardial edema; therefore, a relative decrease in AQP2 expression may represent an adaptive response limiting water accumulation in cardiomyocytes. Although AQP2 is not classically considered a cardiac aquaporin, its ectopic or stress-induced expression in cardiac tissue suggests a context-dependent role in myocardial fluid homeostasis (Verkerk et al. 2019, Shangzu et al. 2022).

AQP3 was first identified in the basolateral membrane of the collecting duct in the kidney. In addition to water transport, AQP3 can also transport glycerol and urea (Koral et al. 2021, Adeoye et al. 2022). There are controversial data in the literature regarding whether AQP3 expression is present in rat hearts (Egan et al. 2006, Rutkovskiy et al. 2013). Specifically, Egan et al. (2006) reported minimal or absent AQP3 expression in rat cardiac tissue, whereas Rutkovskiy et al. (2013) detected low but measurable levels of AQP3 transcripts and protein, suggesting species, method or sensitivity-dependent variability in detection.

However, our study, it was revealed that AQP3 was expressed in rat cardiac tissue, and that the expression was in cardiac myocytes and tubules, and the staining intensity decreased in the treatment groups compared to the control and stress groups. This reduction in AQP3 staining intensity in the treatment groups may indicate a modulatory effect on myocardial water and solute transport, potentially reflecting a protective adaptation that limits cellular swelling or alters energy substrate handling through glycerol transport. These findings suggest that AQP3 may contribute to cardiac osmotic balance and metabolic flexibility under stress conditions, although further quantitative and functional studies are required to confirm its physiological significance in the heart.

It has been reported that water transported by AQP3 can affect many functions related to inflammation, cell proliferation, and cell migration (Papadopoulos et al. 2008, Al-Samir et al. 2016). Increased AQP3 expression can lead to increased water uptake into cells, leading to cell damage (Kwon et al. 2013). Studies have reported that antioxidant agents reduce AQP3 gene expression and protect cells from the toxic effects of water (Dong et al. 2020, Tüfekçioğlu et al. 2023). In this study, we can say that ashwaganhda, with its antioxidant properties, has a protective effect on cell damage and edema caused by stress treatment in cardiac tissue, via AQP3.

In this study, sertraline and ashwagandha were used as comparative interventions. Sertraline, a selective serotonin reuptake inhibitor (SSRI), exerts its effects primarily through central serotonergic modulation and has been shown to reduce stress-related neuroendocrine activation and oxidative stress. In contrast, ashwagandha is considered an adaptogenic herbal compound with multimodal effects, including antioxidant, anti-inflammatory and neuroendocrine regulatory actions (Gokdemir et al. 2025). While both agents appeared to modulate AQP expression and myocardial morphology

to some extent, their mechanisms of action are likely distinct. The comparable trends observed between the sertraline and ashwagandha groups may suggest that both pharmacological and phytotherapeutic approaches can influence stress-induced cardiac changes, although ashwagandha may exert additional peripheral effects through modulation of oxidative stress and cellular hydration mechanisms.

In this study, serum biochemical parameters were evaluated as complementary indicators of myocardial integrity and systemic osmotic balance. Although no statistically significant differences were observed between groups in CK levels, the numerical increase observed in the stress and treatment groups may reflect early or subclinical myocardial stress that has not yet translated into significant systemic biomarker elevation. This interpretation is consistent with the histopathological findings of interstitial edema, hemorrhage and myocardial degeneration. Similarly, slight fluctuations in sodium, potassium and chloride levels may indicate subtle changes in fluid-electrolyte balance under chronic stress conditions. These changes are consistent with the changes observed in aquaporin expression and myocardial water distribution. Taken together, the biochemical, histological, and immunohistochemical findings support the hypothesis that chronic stress affects myocardial osmotic and ionic homeostasis at the tissue level before producing significant systemic biochemical disturbances. It also supports the hypothesis that both pharmacological (sertraline) and phytotherapy (ashwagandha) interventions can partially regulate these changes.

In conclusion, it is thought that there are changes in the expressions of cardiac aquaporins AQP1, AQP2, and AQP3, which have a potential effect on heart pathophysiology, in the depression model induced by mild stress. Sertraline and ashwagandha, when used for treatment, may particularly affect the expression of AQP2 and AQP3. However, more comprehensive and further studies are needed to determine the effects of AQP1, AQP2 and AQP3 on cardiac dysfunctions such as myocardial edema and angiogenesis.

This study has several limitations that should be considered. First, the relatively small sample size limits the statistical power and generalizability of the findings. Second, aquaporin expression was primarily assessed by immunohistochemistry, and quantitative protein or gene expression analyses were not performed. Third, functional cardiac assessments were not included. In addition, although both sertraline and ashwagandha were evaluated, the underlying molecular mechanisms of their comparative effects could not be fully elucidated. Future studies with larger cohorts, quantitative molecular techniques, and integrated functional analyses are needed to better clarify the role of aquaporins under chronic stress conditions and to define the cardioprotective mechanisms and potential translational relevance of ashwagandha.

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Author Declarations

Ethics approvals

All experimental procedures were approved by the Animal Experiments Local Ethics Committee of Dicle University Prof. Dr. Sabahattin Payzın Health Sciences Application and Research Center (Approval No: 2-06/09).

Use of generative artificial intelligence

No generative artificial intelligence (AI) tools were used in the preparation of this manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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