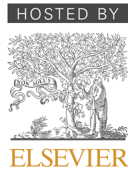
Saudi Pharmaceutical Journal 32 (2024) 102105



Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Diuretic effects of Hecogenin and Hecogenin acetate via aldosterone synthase inhibition

Abdulmohsin J. Alamoudi [a], Maria Nazeer [b], Nabi Shah [b,*], Saif Ullah [b], Meshal Alshamrani [c], Waleed Y. Rizg [d], Osama M. Ashour [a], Ashraf B. Abdel-naim [a], Abdul Jabbar Shah [b]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, King Abdulaziz University, Jeddah, 21589, Saudi Arabia
[b] Pharmacogenetics Research Lab, Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, Pakistan
[c] Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan 45142, Saudi Arabia
[d] Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia

ARTICLE INFO

Keywords:
Hecogenin
Hecogenin acetate
Spironolactone
Furosemide
Aldosterone synthase
Healthcare

ABSTRACT

Hecogenin (HEC) is a steroidal saponin found in many plant species and serves as a precursor for steroidal drugs. The diuretic effects of HEC and its derivative, hecogenin acetate (HA), remain largely unexplored. The present study aimed to explore the potential diuretic effects of HEC and HA compared to furosemide (FUR) and spironolactone (SPIR). Additionally, the study aimed to explore the underlying mechanism particularly focusing on aldosterone synthase gene expression. Fifty-four Sprague-Dawley rats were allocated into nine groups (Group 1–9). Group 1 (control) received the vehicle, Groups 2 received FUR 10 mg/kg, Group 3, 4, and 5 were given HEC, while Groups 6, 7 and 8 received HA i.p at doses of 5, 10, and 25 mg/kg, respectively. Group 9 received SPIR i.p at the dose of 25 mg/kg. Urine volume, diuretic index and diuretic activity were monitored at 1, 2, 3, 4, 5, 6, and 24 h post-administration. Treatment was given daily for seven days. After that, rats were sacrificed and blood was collected for serum electrolytes determination. Adrenal glands were dissected out for gene expression studies. The results revealed that HEC and HA at the administered doses significantly and dose-dependently increased urine and electrolyte excretion. These results were primarily observed at 25 mg/kg of each compound. Gene expression studies demonstrated a dose-dependent reduction in aldosterone synthase gene expression, suggesting aldosterone synthesis inhibition as a potential mechanism for their diuretic activity. Notably, HA exhibited more pronounced diuretic effects surpassing those of HEC. This enhanced diuretic activity of HA can be attributed to its stronger impact on aldosterone synthase inhibition. These findings offer valuable insights into the diuretic effects of both HEC and HA along with their underlying molecular mechanisms.

1. Introduction

Diuretics are the class of medications aimed at increasing the water and electrolytes excretion from the body, play a crucial role in managing conditions associated with fluid retention (Titko et al., 2020, Yang et al., 2021). These conditions include edema, hypertension, and congestive heart failure, where maintaining an optimal fluid balance is pivotal for therapeutic success (Novak and Ellison, 2022, Roush et al., 2013). In hypertension treatment, diuretics are applied either alone or in combination with other antihypertensive drugs to reduce cardiovascular and cerebrovascular complications and mortality rates (Melka et al., 2016, Welu et al., 2020). Their response is often influenced by specific pathways regulating water and electrolyte homeostasis. The renin-angiotensin-aldosterone system (RAAS) stands out as a primary pathway responsible for sustaining fluid and electrolyte balance, with aldosterone serving as a critical mediator in sodium reabsorption. Aldosterone, a steroid secreted from the zona glomerulosa cells of the adrenal cortex, primarily acts on the kidneys to enhance sodium and water reabsorption while promoting potassium excretion (Alfie, 2023, Amatruda et al., 2022). The regulation of aldosterone production entails several enzymatic processes, including the conversion of pregnenolone from cholesterol and the transformation of corticosterone to aldosterone

* Corresponding author at: Pharmacogenetics Research Lab, Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, Pakistan.
E-mail addresses: ajmalamoudi@kau.edu.sa (A.J. Alamoudi), marianazeer90@gmail.com (M. Nazeer), nabishah@cuiatd.edu.pk (N. Shah), saifullahsarim@gmail.com (S. Ullah), malshamrani@jazanu.edu.sa (M. Alshamrani), wrizq@kau.edu.sa (W.Y. Rizg), ommohamed@kau.edu.sa (O.M. Ashour), aaabdulalrahman1@kau.edu.sa (A.B. Abdel-naim), jabbarshah@cuiatd.edu.pk (A.J. Shah).

https://doi.org/10.1016/j.jsps.2024.102105
Received 9 March 2024; Accepted 15 May 2024
Available online 16 May 2024
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by an enzyme aldosterone synthase, encoded by the CYP11B2 gene (Alvarez-Madrazo et al., 2013). The amino acid sequence of CYP11B2 shares approximately 93 % similarity with CYP11B1, which encodes the 11β-hydroxylase enzyme. The CYP11B2 gene undergoes intron conversion in intron 2, where a portion of the wild-type intron 2 is substituted by the similar region of intron 2 from the CYP11B1 gene. This conversion in CYP11B2 could potentially affect aldosterone production and thereby influence the renal handling of electrolytes (Zhang et al., 2010). Another important gene, CYP17A1, plays a crucial role in synthesizing steroidogenic enzyme and catalyzes the metabolic conversion from pregnenolone to 17-alpha hydroxy-pregnenolone, an intermediate substance crucial in the biosynthesis of cortisol and sex steroids. In contest with this synthetic route, pregnenolone undergo metabolism that ultimately leading to the production of aldosterone (Huber et al., 2015, Li et al., 2013).

Saponins are known for their various biological activities affecting the nervous, cardiovascular and gastrointestinal systems, as well as their involvement in inflammatory and infectious conditions (Borges et al., 2023). Hecogenin (HEC) is a steroidal saponin found in plants of the Agave and Tribulus terrestris L. genus (Ingawale, 2020). It acts as a precursor in steroidal drug synthesis, and is naturally present as a byproduct of plant metabolism, mainly in the older leaves of Agave sisalana (Borges et al., 2023). Similarly, HEC acetate derivative a chemically-modified form, hecogenin acetate (HA). Both compounds possess potential pharmacological implications, including antitumor (Liagre et al., 2007), anti-inflammatory (Ingawale and Patel, 2016) Gastroprotective, antiulcerogenic (Santos Cerqueira et al., 2012, Sousa et al., 2023), anti-hyperalgesic (Quintans et al., 2014, Santos Passos et al., 2021) and antinociceptive (Carvalho et al., 2017) effect. The literature reports cardioprotective effect (Pei et al., 2022). However, there is a lack of information regarding their possible potential diuretic properties and their impact on aldosterone synthase gene expression. Due to their cardioprotective role through ion channel modification and reducing intracellular calcium, these compounds might also have diuretic effects. By reducing intracellular calcium in cardiac tissue to mitigate contractility, they might impact the kidneys. Based on the anti-hypertensive effects of these compounds observed in folk medicine, it was hypothesized that these actions involve modification aldosterone synthesis and/or expression. Therefore, the objective of this investigation was to conduct a comparative evaluation of the potential diuretic action of HEC and HA as well as their influence on aldosterone synthase gene expression.

2. Methodology

2.1. Animals

Fifty four Sprague Dawley rats (180 to 240 g) were selected six days before the experiment and maintained under ideal conditions, with unrestricted access to food and tap water, while adhering to a 12-hour light/dark cycle. Experimental procedures were conducted in accordance with the Guidelines for Care and Use of Laboratory Animals (National Research Council, 2011). Approval for all experimental procedures was obtained from the departmental research committee review board with issue no of PHM.Eth/CS-M02-02–2824.

2.2. Chemicals

HEC, HA, furosemide (FUR), spironolactone (SPIR), pentobarbital sodium and DMSO were purchased from Merck & Co., Inc. (Rahway, NJ, USA).

2.3. Preliminary study

In the initial assessments, rats received 1 mL/100 g distilled water. Subsequently, they were individually housed in metabolic cages for urine collection and the volumes were measured after 6 h. Rats excreting at least 40 % of the dosing volume were included in the study, while those with less than 40 % excretion were excluded. The included rats were randomly assigned into nine groups (6 rats each) and underwent a 7-day acclimatization period in individual metabolic cages (Ntchapda et al., 2014).

2.4. Acute diuretic study

Rats were assigned to nine groups, six animals in each. Following an overnight fast with unrestricted access to water, the 1st group received distilled water 1 mL/100 g and considered as control. The 2nd group received an i.p dose of FUR (10 mg/kg). The 3rd, 4th and 5th groups were administered HEC i.p. at doses of 5, 10 and 25 mg/kg, respectively. The HA at the dose of 5, 10 and 25 mg/kg, i.p was administered in 6th, 7th and 8th groups respectively. The 9th group received SPIR i.p (25 mg/kg). After 1, 2, 3, 4, 5, 6 and 24 h, urine was measured and collected (Hullatti et al., 2011, Yang et al., 2021).

2.5. Sub-acute diuretic study

The sub-acute diuretic activity all rats were treated for 7 days. Body weight, urine volume, pH and electrolyte concentrations were recorded. After that, pentobarbital sodium at a dose of 40 mg/kg was administered IP to anesthetize the rats and blood samples were obtained via cardiac puncture (Liu et al., 2019). The blood was then centrifuged at 2500 rpm at 4 °C for 15 min and serum was extracted. Urine samples were stored at −20 °C while serum samples were stored at −80 °C for subsequent analysis (Yang et al., 2021).

2.6. Determination of diuretic index, activity and electrolytes' concentrations

After drug administration, urine volume was assessed at 1, 2, 3, 4, 5, 6 and 24 h. The pH was determined using a digital pH meter form fresh urine sample. Urine and serum electrolyte concentrations (potassium (K), sodium (Na) and chloride (Cl) along with serum urea and creatinine were assessed using an automatic analyzer (Beckman Coulter AU480 wISE Chemistry Analyzer and Diamond SOP23-0005F Prolyte analyzer). The diuretic activity and diuretic action were calculated from urine electrolytes. Diuretic activity was categorized as 'nil,' 'little,' 'moderate,' and 'good' if the values fell within the ranges < 0.72, 0.72–1.00, 1.00–1.5, and > 1.5, respectively (Al-Saikhan & Ansari, 2016). Saluretic and natriuretic effects as well as carbonic anhydrase inhibition were assessed using following formulas (M K and Amoghimath, 2018; Welu et al., 2020).

$$Urinaryexcretion = \frac{Totalurinaryoutput}{Totalvolumeofliquidadministered} \times 100$$

$$Diuretic\ action = \frac{Urinary\ excretion\ of\ the\ test\ group}{Urinary\ excretion\ of\ the\ control\ group}$$

$$Diureticindex = \frac{Diureticactionofthetestgroup}{Diureticactionofthestandardgroup}$$

$$Saliureticindex = \frac{UrinaryNa^{+}, K^{+}, Cl^{-} levelintestgroup}{UrinaryNa^{+}, K^{+}, Cl^{-} levelincontrolgroup}$$

$$Natriureticindex = \frac{UrinaryNa^{+} levelinthesametestgroup}{UrinaryK^{+} levelinthesametestroup}$$

$$CAIindex = \frac{UrinaryCl^{-} levelinthesametestgroup}{SumofUrinaryNa^{+} andK^{+} levelinthesametestgroup}$$

Table 1
Primers Sequence of CYP 11B2, CYP 11B1, and CYP 17A1.

Gene	Accession No	Primers Sequence (5′>3′)
GAPDH	NM_017008.4	Forward primer: CCGCATCTTCTTGTGCAGTG Reverse primer: ACCAGCTTCCCATTCTCAGC
CYP 11B2	NM_012538.2	Forward primer: CATTGTGGCAGCACTAATAACT Reverse primer: GGCATATAGCGCTCAGGTCTT
CYP 11B1	XM_039079756.1	Forward primer: GGGCCAAGAGAACCTACACC Reverse primer: CCAGTCTGCCCCATTTAGCA
CYP 17A1	NM_012753.3	Forward primer: GTGCAGGGAGAAGTTCGACA Reverse primer: AAGCCAGGATCCACTTGAGC

GAPDG = Glyceralde-hyde 3-phosphate dehydrogenase, CYP 11 B2 = Aldosterone synthase,
CYP 11B1 = 11β hydroxylase, CYP 17A1 = 17α-hydroxylase.

2.7. Gene expression analysis

Total RNA was isolated from the tissue of adrenal gland using RNA isolation reagent, following the manufacturer's instructions (Thermo fisher scientific, catalog no: 15–596-018). The adrenal glands from each group were homogenized in autoclaved Eppendorf tubes with 0.5–1 mL of TRIzol reagent using a Benchmark Scientific homogenizer. Addition of chloroform (200–400 μL) and centrifugation (13,000 rpm for 10–15 min at 4 °C) resulted in three distinct layers: an upper RNA layer, a middle DNA or protein layer and a lower organic layer. The upper RNA layer was carefully shifted to a fresh Eppendorf tube, and isopropanol (500–550 μL) was added. Subsequent centrifugation (14,000 rpm for 15–20 min at 2–8 °C) formed RNA pellets at the bottom of the tube. The washing of RNA pellet was done with 1 mL of 75 % ethanol, followed by air-drying for 5–10 min. Nuclease-free water 50 μL was introduced into each tube and RNA samples were kept at −20 °C. Purity (A260/A280) and concentration (ng/μL) were determined using NanoDrop UV/VIS spectrophotometer (Bo et al., 2021; García-Alegría et al., 2020). Following RNA extraction, cDNA synthesis was performed using cDNA synthesis kit following the manufacturer's instructions (Thermo fisher Scientific, catalog no: K1622).

To analyze mRNA relative expression, a PCR reaction mixture was prepared using DreamTaq Green PCR Master mix (Thermo fisher Scientific, catalog no: K1081). The mixture included diluted cDNA, gene-specific primers, nuclease-free water and master mix, with the final volume adjusted to 20 μL. Primer sequences for specific genes is listed in Table 1. GAPDH was used as the housekeeping gene for accurate normalization. PCR was conducted with thermal cycling parameters: an initial denaturation at 95 °C for 5 min in the first cycle then 95 °C for 30 s for the rest of cycles. The annealing step involved different temperatures according to genes: 58 °C for CYP11B2, 59 °C for CYP11B1, and 57 °C for CYP17A1 each lasting 30 s. The extension step lasted for 1 min at 72 °C, subsequent to a final extension lasting 5 min at the same temperature. mRNA expression was visualized through gel electrophoresis, with a gel prepared by mixing 2 gm of agarose in 1x TBE buffer (100 mL). After heating and cooling at room temperature, 5–8 μL of Cyber Safe (catalog no: S33102) was added. Gel electrophoresis ran for 25–30 min at 120 V, and PCR bands were observed under UV light for gene expression (Lee et al., 2012). For the normalization of gene expression data, the GAPDH housekeeping gene was used. The quantification of gene expression levels was performed three times for each band using ImageJ software.

2.8. Data analysis

The data expressed as mean values ± standard deviation, and statistical analysis was carried out through the one-way analysis of variance. The differences among all groups were analyzed using Tukey's test in GraphPad Prism 8 software (San Diego, CA, USA). Values are considered significant when their corresponding *p*-values are less than 0.05.

3. Results

3.1. Effect of HEC and HA on body weight

The body weight of each rat was measured on the first day (initial weight) and day 7 (final weight). FUR administration resulted in weight loss, while the low-dose of HEC showed a steady increase in weight similar to the control group. However, the higher doses of HEC caused reduction in body weight. In contrast, HA at all used doses and SPIR demonstrated a non-significant decrease in weight compared to FUR as depicted in Fig. 3.1 **(A) and (B).**

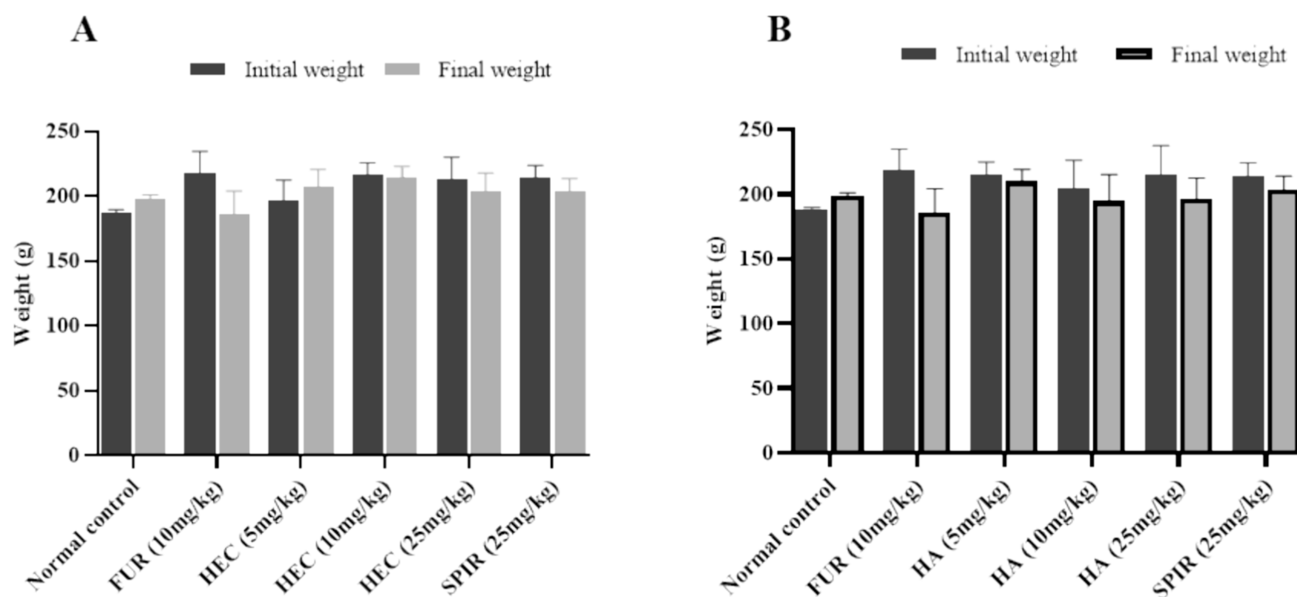

Fig. 3.1. Effect of hecogenin (HEC; 5, 10, and 25 mg/kg) (Fig. A) and hecogenin acetate (HA; 5, 10, and 25 mg/kg) (Fig. B), and the reference drugs furosemide (FUR; 10 mg/kg) and spironolactone (SPIR; 25 mg/kg) on body weight at days 1 and 7. Data are expressed as mean ± SD (n = 3–6).

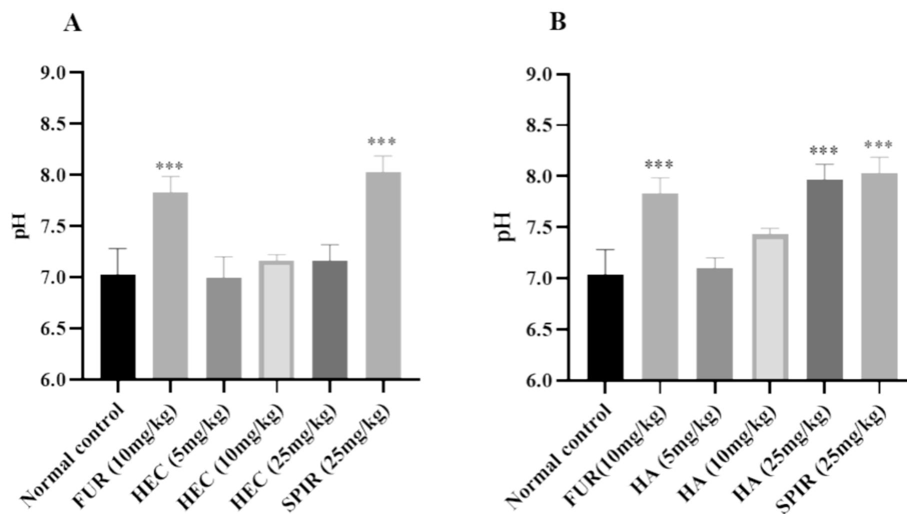

Fig. 3.2. Effect of hecogenin (HEC; 5, 10, and 25 mg/kg) (A) and hecogenin acetate (HA; 5, 10, and 25 mg/kg) (B), and the reference drugs furosemide (FUR; 10 mg/kg) and spironolactone (SPIR; 25 mg/kg) on urine pH. The measurements were taken from 24-hour urine samples, and values are presented as mean ± SD (n = 3–6). Significance level is represented as $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, determined through one-way ANOVA.

Table 2
Effect of HEC, HA, FUR and SPIR on urine output (ml) at 1, 2, 3, 4, 5, 6, and 24 h post administration.

Treatment	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	6 Hours	24 Hours
Control	0.93 ± 0.25	1.70 ± 0.10	2.50 ± 0.10	3.0 ± 0.10	3.50 ± 0.10	4.20 ± 0.10	5.60 ± 0.10
FUR (10 mg/kg)	2.23 ± 0.25***	3.80 ± 0.36***	5.70 ± 0.17***	6.63 ± 0.15***	7.70 ± 0.20***	9.30 ± 0.61***	10.83 ± 0.65***
HEC (5 mg/kg)	0.80 ± 0.10	1.3 ± 0.10	2.83 ± 0.11	3.06 ± 0.05	3.80 ± 0.11	4.80 ± 0.10	5.83 ± 0.05
HEC (10 mg/kg)	1.13 ± 0.05	2.26 ± 0.05	3.53 ± 0.15***	4.0 ± 0.10***	4.73 ± 0.11***	5.30 ± 0.10***	6.36 ± 0.15**
HEC (25 mg/kg)	1.60 ± 0.20**	2.93 ± 0.30 ***	3.80 ± 0.10***	4.96 ± 0.25 ***	5.26 ± 0.15***	6.10 ± 0.10 ***	6.93 ± 0.11***
HA (5 mg/kg)	1.60 ± 0.10***	2.70 ± 0.10***	3.50 ± 0.10***	4.56 ± 0.15***	5.66 ± 0.15***	5.96 ± 0.15***	7.46 ± 0.15***
HA (10 mg/kg)	1.83 ± 0.05***	2.8 ± 0.10***	3.03 ± 0.05***	5.63 ± 0.05***	6.30 ± 0.10***	7.86 ± 0.05***	9.30 ± 0.10***
HA (25 mg/kg)	2.06 ± 0.05***	4.10 ± 0.30***	5.53 ± 0.32***	6.53 ± 0.20***	7.50 ± 0.10***	9.13 ± 0.61***	10.3 ± 0.76***
SPIR (25 mg/kg)	2.36 ± 0.15***	3.60 ± 0.17***	5.30± 0.10***	6.16 ± 0.15***	7.03 ± 0.05***	8.10 ± 0.26***	9.43 ± 0.37***

Results are expressed as mean ± standard deviation (SD). n = 3–6.
FUR: Furosemide HEC: Hecogenin HA: Hecogenin Acetate SPIR: Spironolactone.

Table 3
Effect of HEC and HA on diuretic index and activity.

Drug (mg/kg)	After 6 h			After 24 h		
	Diuretic action/ Diuretic index	**Diuretic activity/ Lipschitz value FUR**	**Diuretic activity/ Lipschitz value SPIR**	**Diuretic action/ Diuretic index**	**Diuretic activity/ Lipschitz value (FUR)**	**Diuretic activity/ Lipschitz value (SPIR)**
Control	1	–	–	1	–	–
FUR (10 mg/kg)	2.21	1	1.14	1.93	1	1.14
HEC (5 mg/kg)	1.14	0.51	0.59	1.04	0.53	0.61
HEC (10 mg/kg)	1.26	0.56	0.65	1.13	0.58	0.67
HEC (25 mg/kg)	1.45	0.65	0.73	1.23	0.63	0.73
HA (5 mg/kg)	1.41	0.64	0.73	1.33	0.68	0.79
HA (10 mg/kg)	1.87	0.84	0.97	1.66	0.85	0.98
HA (25 mg/kg)	2.17	0.98	1.12	1.83	0.95	1.09
SPIR (25 mg/kg)	1.92	0.87	1	1.68	0.87	1

Results are normalized to control, n = 3–6.
FUR: Furosemide HEC: Hecogenin HA: Hecogenin Acetate SPIR: Spironolactone.
Diuretic action = urine volume of test group/urine volume of control group.
Diuretic activity = urine volume of test group/urine volume of standard group.

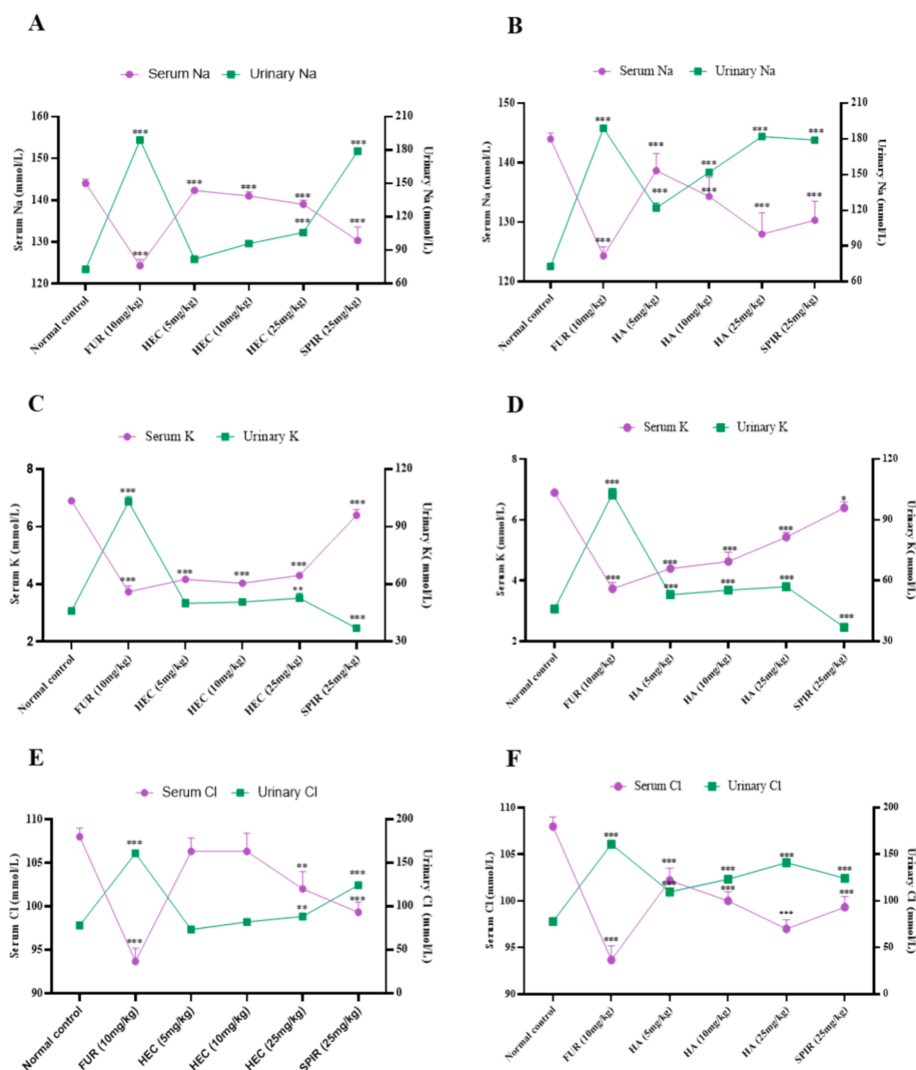

Fig. 3.3. Effect of HEC and HA on urinary and serum sodium concentrations (A and B), urinary and serum K (C and D) urinary and serum Cl (E and F). Data expressed as mean ± SD, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicates a significant level compared to the control group.

3.2. Effect of HEC and HA on urinary pH

Urinary pH of rats administered HEC and HA was evaluated using 24-hour urine samples. The baseline urinary pH for control rats was recorded as 7.0 ± 0.25. Following administration of 5, 10, and 25 mg/kg HEC, the urine pH levels were measured at 7.0 ± 0.20, 7.167 ± 0.05, and 7.167 ± 0.15, respectively as depicted in Fig. 3.2 **(A)**. For HA administration at doses of 5, 10, and 25 mg/kg body weight, the corresponding urine pH values were 7.1 ± 0.10, 7.4 ± 0.05, and 7.9 ± 0.15 as shown in Fig. 3.2 **(B)**. In comparison, the standard drugs FUR and SPIR exhibited urine pH values of 7.8 ± 0.15 and 8.0 ± 0.15, respectively. While HEC did not induce any changes in urinary pH, higher doses of HA resulted in a significant increase in pH ($p < 0.001$), rendering the urine alkaline. This alteration was statistically significant compared to the normal pH level and resembled the effects observed with the standard drugs.

3.3. Effect of HEC and HAC on urine output, diuretic index and activity

At a low dose of 5 mg/kg, HEC exhibited no significant alteration in urine output compared to the control group at all the time points while at 10 mg/kg it demonstrated a significant rise in urine volume after two hours. Similarly, HEC at the higher dose (25 mg/kg), increased urine volume at all the time points (p-value < 0.001). In contrast, HA at all doses (5, 10, and 25 mg/kg) led to a highly significant rise in urine volume (p-values < 0.001), as shown in Table 2.

HEC at the doses of 5, 10 and 25 mg/kg exhibited a moderate diuretic response and HA at the dose of 5 mg/kg depicts moderate diuretic activity. While HA at 10 mg/kg and 25 mg/kg demonstrated a good diuretic activity as shown in Table 3.

3.4. Effect of HEC, HA, FUR and SPIR on urinary and serum electrolytes

HEC at all doses did not show any significant change in serum concentration of sodium, while significantly increased urinary sodium (p-values < 0.001) (Fig. 3.3 **(A) and (B)**). HA, at all doses, caused a highly significant increase in urinary sodium while serum sodium was significantly decreased at 25 mg/kg.

Regarding urinary potassium (K), HEC at all doses showed no significant effects on urinary K, while at the dose of 25 mg/kg, it showed a less significant increase in urinary K (p-value = 0.006). On the other hand, HA, at all doses, significantly decreased serum K values, while at 5 mg/kg causes a less significant increase in urinary K and at the doses of 10 and 25 mg/kg, it causes a highly significant increase in urinary K (Fig. 3.3 **(C) and (D)**). Urinary chloride (Cl) excretion was significantly increased, and serum Cl was significantly decreased by HA at all doses. HEC at 5 and 10 mg/kg showed no effect on urinary and serum Cl, while at high dose, however, it causes a less significant effect on both urinary

Table 4
Effect of HEC and HA on saluretic, natriuretic and CAI index.

Drug (mg/kg)	Na^+ Index	K^+ Index	Cl^- index	Sal uretic effect (Na^++Cl^-)	Natriuretic effect (Na^+/k^+)	$Na^+ + K^+$	CAI activity $Cl^-/(Na^+ + K^+)$	Sal uretic index	Natriuretic index	CAI index
Control	1	1	1	151	1.58	119	0.65	1	1	1
HEC (5 mg/kg)	1.12	1.08	0.93	155	1.64	132	0.55	1.0	1.03	0.84
HEC (10 mg/kg)	1.31	1.10	1.05	178	1.88	147	0.55	1.17	1.18	0.85
HEC (25 mg/kg)	1.45	1.13	1.12	194	2.03	158	0.55	1.28	1.28	0.84
HA (5 mg/kg)	1.67	1.15	1.39	231	2.30	175	0.62	1.52	1.45	0.95
HA (10 mg/kg)	2.08	1.19	1.57	275	2.76	207	0.59	1.82	1.74	0.90
HA (25 mg/kg)	2.49	1.23	1.80	323	3.19	239	0.58	2.13	2.01	0.90
FUR (10 mg/kg)	2.5	2.2	2.06	492	1.83	292	0.55	3.25	1.15	0.84
SPIR (25 mg/kg)	2.4	0.80	1.58	303	4.83	216	0.57	2.00	3.04	0.87

Results are normalized to the mean of control n = 3–6.
FUR: Furosemide HEC: Hecogenin HA: Hecogenin Acetate SPIR: Spironolactone.
CAI: carbonic anhydrase inhibition
Na + index: sodium excretion in test group/sodium excretion in control group.
K + index: potassium excretion in test group/potassium excretion in control group.
Cl − index: chloride excretion in test group/chloride excretion in control group.
Saluretic index: saluretic activity in test group/saluretic activity in control group.
Natriuretic index: natriuretic activity in test group/natriuretic activity in control group.
Carbonic anhydrase inhibition index: CAI activity in test group/CAI activity in control group.

Cl and serum Cl decrease as shown in Fig. 3.3 **(E) and (F)**.

3.5. Effect of HEC and HA on the saluretic, natriuretic and CAI indices

The electrolyte, saluretic, natriuretic, and CAI index values are presented in Table 4**.** HA, 5 mg/kg and 10 mg/kg, showed good saluretic activities, while at 25 mg/kg, it displayed a robust saluretic activity with a value of 2.13, compared to the control group. Meanwhile, HEC showed a less pronounced saluretic effect. Natriuretic values less than 2 indicate satisfactory natriuresis, while values above 2 indicate favorable natriuresis. Specifically, HA at doses of 5 mg/kg and 10 mg/kg, as well as all doses of HEC, demonstrated satisfactory natriuretic effects. However, at the 25 mg/kg, HA indicated favorable natriuresis with a value of 2.01. CAI values less than 0.8 indicate strong CAI activity, but all doses of HEC and HA exceeded 0.8, suggesting a lack of carbonic anhydrase inhibition for these compounds.

3.6. Effect of HEC and HA on serum urea and creatinine

Serum urea and creatinine were also assessed. HEC, 5 and 10 mg/kg, did not induce a statistically significant decrease in serum urea and creatinine values, while at 25 mg/kg, it exhibited moderate effects (p-value of 0.01) as shown in Fig. 3.4 **(A) and (B)**. In contrast, HA significantly decreased both serum urea and creatinine at all doses. However, the extent of the decrease induced by HA was not as pronounced as observed with furosemide, suggesting that HA may have a lesser impact on kidney function as shown in Fig. 3.4 **(B) and (D)**.

3.7. Effect of HEC and HA on gene expression

The effects of HEC and HA on the relative fold expression of CYP11B2, CYP11B1, and CYP17A1 was assessed based on their respective bands, as illustrated in Fig. 3.5. At 5 mg/kg and 10 mg/kg, HEC did not show a significant decrease in CYP11B2 gene expression. However, at the 25 mg/kg, it significantly decreased the gene expression (p value = 0.047) as depicted in Fig. 3.5 **(A)**. At 5 and 10 mg/kg doses, HEC did not exhibit significant inhibition of CYP11B1 expression, while at 25 mg/kg, it induced significant inhibition (p value = 0.016) as depicted in Fig. 3.5 **(C)**. In contrast, HA at all doses significantly inhibited both CYP11B2 and CYP11B1 gene expression, as shown in Fig. 3.5 **(B) and (D)**. Furthermore, HEC at 5 mg/kg and 10 mg/kg did not significantly affect CYP17A1 expression, however at a higher dose (25 mg/kg) it led to a significant decrease (p value = 0.012). Consistently, HA at all doses used in this study caused a highly significant decrease in CYP17A1 expression, as illustrated in Fig. 3.5 **(E) and (F)**.

4. Discussion

Saponin, known for their diverse biological activities, exhibit effects on renal electrolyte excretion (Amuthan et al., 2012). Among these, HEC, a steroidal saponin, acts as a precursor for steroidal drugs and is found naturally in older leaves of Agave sisalana (Ingawale and Patel, 2016). Its derivative, HA, represents a chemically modified form with promising pharmacological effects, including antitumor, anti-inflammatory, gastroprotective, antiulcerogenic, anti-hyperalgesic, and antinociceptive properties (Borges et al., 2023). Despite these known effects, information regarding their diuretic properties and impact on aldosterone production genes remains limited.

In this study, we assessed the diuretic effects of HEC and HA in comparison to the effects of FUR (a high-ceiling loop diuretic) and SPIR (an aldosterone antagonist) (Lahlou et al., 2007). Both HEC and HA significantly increased urine volume compared to the control group under acute and sub-acute treatments. The diuretic index revealed moderate diuretic effects for HEC (1.45 after 6 h and 1.23 after 24 h) and a more potent diuretic potential for HA (2.17 after 6 h and 1.83 after 24 h) at a dose of 25 mg/kg of each. Evaluation of electrolyte levels to elucidate the diuretic mechanism indicated significant increases in urine Na^+, Cl^-, and K^+ levels for both HEC and HA, accompanied by slight alkalization of urine compared to the control group. The Na^+/K^+ ratio suggested satisfactory natriuresis for HEC (1.28) and favorable natriuresis for HA (2.01), without excessive K + loss. The saluretic effect demonstrated favorable natriuresis for HEC and significant natriuresis for HA, similar to FUR. Notably, both HEC and HA showed increased excretion of Na^+ and Cl^-, while K^+ levels remained relatively stable, indicating enhanced NaCl excretion and K^+ retention. Our findings provide compelling evidence supporting the diuretic effects of HEC and HA, suggesting their potential as diuresis through aldosterone inhibition similar to SPIR. Aldosterone a pivotal hormone in the RAAS pathway, regulates sodium excretion and potassium retention. This suggests that HEC and HA may offer promising diuretic treatments, targeting

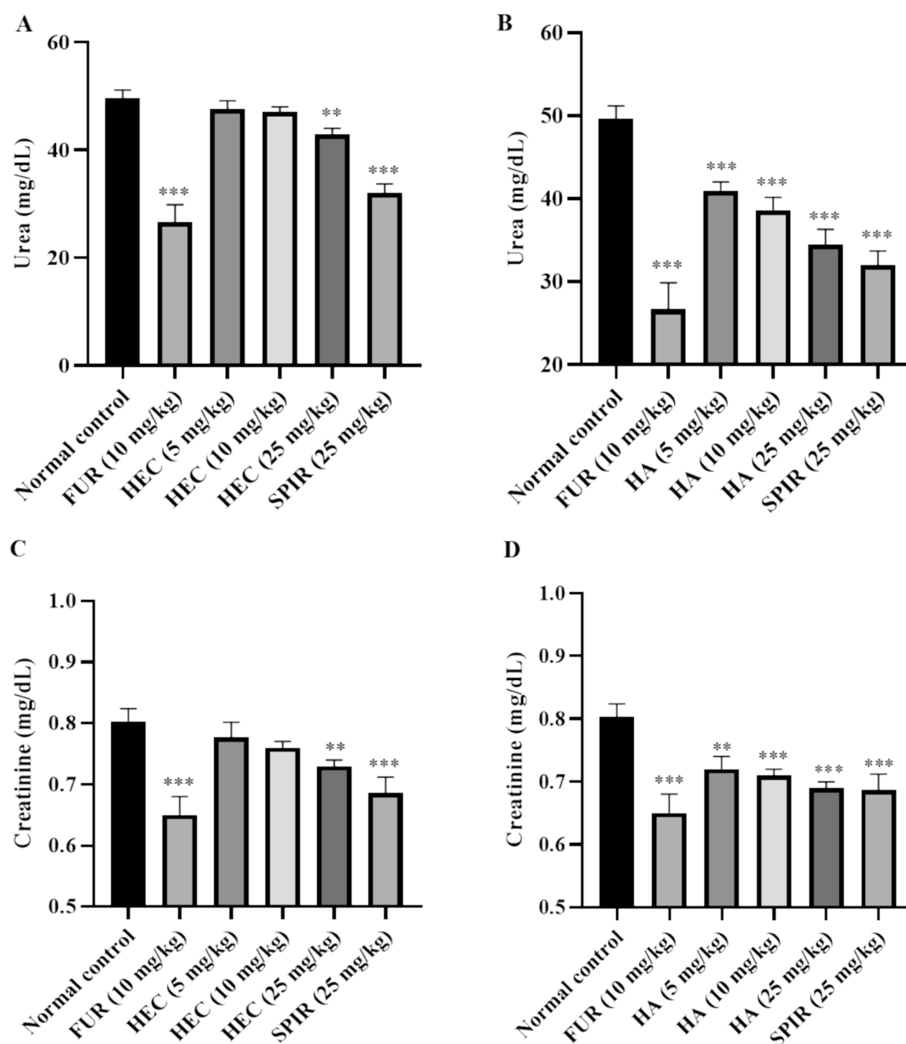

Fig. 3.4. Effect of HEC and HA on serum urea (4A and 4B) and serum creatinine (4C and 4D). Values are presented as mean ± SD (n = 3–6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicates a notable significance difference in comparison to the control group.

aldosterone to maintain fluid and electrolyte balance.

Furthermore, our study aimed to investigate whether these compounds have an effect on the expression of aldosterone synthase gene. To elucidate the underlying mechanism, we conducted gene expression study focusing on CYP 11B2 gene. CYP 11B2 encodes for aldosterone synthase, the enzyme responsible for the final steps in aldosterone biosynthesis. Upregulation of CYP 11B2 increases aldosterone production, potentially resulting in hypertension and electrolyte imbalance (Shah et al., 2023). HEC and HA administered at doses of 5, 10, and 25 mg/kg) exhibited a significant and dose dependent reduction in CYP 11B2 gene expression in contrast to the control group ($p < 0.001$). This suggests that the activity of HEC and HA, which is achieved through the reduction of aldosterone synthesis, modulates fluid and electrolyte balance.

In addition to the observed effects on CYP11B2 gene expression, our study also revealed significant findings regarding gene expression of CYP11B1, which encodes for 11β-hydroxylase enzyme which is crucial in the synthesis of cortisol and corticosterone. Notably, the homology shared between CYP11B1 and CYP11B2 underscores potential similarities in their regulatory mechanisms, emphasizing the relevance of the findings in understanding the hormonal balance and cardiovascular health (Zhang et al., 2010). These insights further underscore the potential significance of HEC and HA in modulating cardiovascular health through their effects on hormone synthesis pathways (Huang et al., 2022). Downregulation of CYP11B1 gene expression by HEC and HA suggests their non-selective inhibitory effects, potentially may impact metabolic and inflammatory pathways by interfering with cortisol and corticosterone synthesis. Additionally, CYP17A1 which encodes for 17α-hydroxylase and 17,20-lyase, contributes to steroid production in both the adrenal glands and reproductive organs (Wróbel et al., 2023). CYP17A1 affects blood pressure indirectly by influencing multiple physiological systems, including the autonomic nervous system, endothelial function, and RAAS. Generally, androgens are linked to elevated blood pressure (Van Woudenberg et al., 2015). Our findings indicated a substantial decrease in CYP17A1 gene expression following administration of both HEC and HA. This suggests that these compounds have an inhibitory effect on the CYP17A1 activity, which plays a crucial role in steroidogenesis. Such downregulation could have implications for various physiological processes influenced by steroid hormones produced by the adrenal glands and reproductive organs. The observed downregulation of steroid synthesis genes by these HEC and HA hints at their potential dual role in diuresis and aldosterone synthase gene inhibition, offering a promising avenue for managing cardiovascular and metabolic disorders.

5. Conclusion

In conclusion, both HEC and HA exhibit significant diuretic effects,

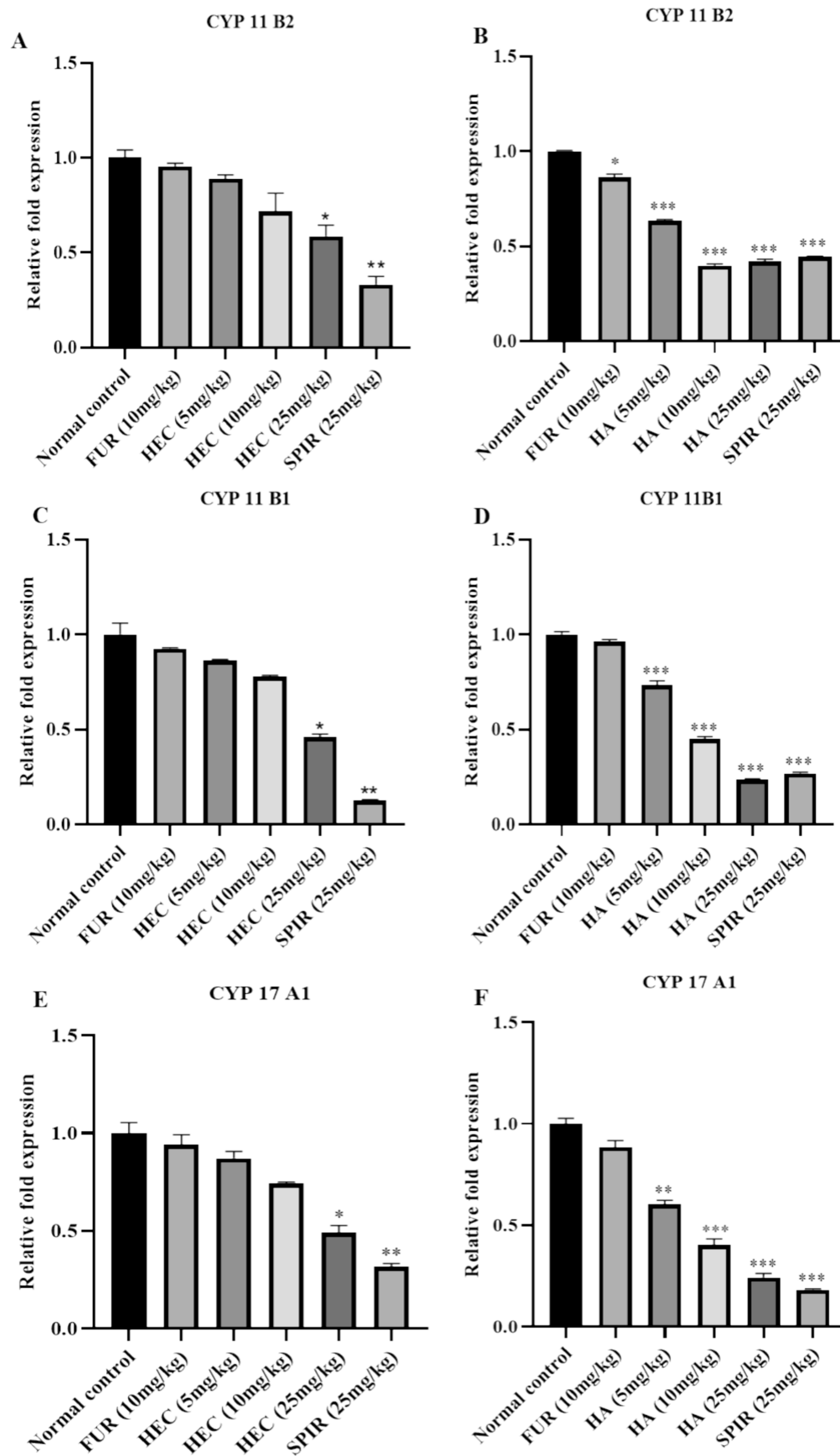

Fig. 3.5. Effect of HEC and HA on expression of CYP 11B2, CYP 11B1, and CYP 17A1. Expression bands and respective graphs illustrating HEC CYP 11B2 (A), HA CYP 11B2 (B), HEC CYP 11B1 (C), and HA CYP 11B1 (D), HEC CYP 17A1 (E), and HA CYP 17A1 (F) are presented. The reported values represent the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001) shows a significant levels compared to the control group.

akin to the standard drug SPIR, suggesting a similar mechanism of action. Additionally, gene expression studies indicate that both HEC and HA down regulate the CYP11B2 and CYP11B1, with the potential to modulate fluid and electrolyte balance, metabolic pathways, and steroid hormone regulation. Notably, HA demonstrates more pronounced these effects than HEC. Further research is needed to fully understand their pharmacokinetic profile, their mechanisms of action and their clinical implications in diseased state.

Funding.

The Deanship of Scientific Research (DSR) at King Abdulaziz University (KAU), Jeddah, Saudi Arabia has funded this Project under grant no (G:522–166-1443) .

CRediT authorship contribution statement

Abdulmohsin J. Alamoudi: Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Maria Nazeer:** Formal analysis, Investigation, Methodology, Writing – original draft. **Nabi Shah:** Conceptualization, Data curation, Formal analysis, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing. **Saif Ullah:** Formal analysis, Software, Writing – review & editing. **Meshal Alshamrani:** Resources, Validation, Writing – review & editing. **Waleed Y. Rizg:** Resources, Validation, Visualization, Writing – review & editing. **Osama M. Ashour:** Resources, Validation, Writing – review & editing. **Ashraf B. Abdel-naim:** Resources, Visualization, Writing – review & editing. **Abdul Jabbar Shah:** Conceptualization, Investigation, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Alfie, J., 2023. Aldosterone breakthrough from a pharmacological perspective. Hypertens. Res. 46 (5), 1350. https://doi.org/10.1038/s41440-023-01204-2.

Al-Saikhan, F.I., Ansari, M.N., 2016. Evaluation of the diuretic and urinary electrolyte effects of methanolic extract of Peganum harmala L. in Wistar albino rats. Saudi J Biol Sci. 23 (6), 749–753. https://doi.org/10.1016/j.sjbs.2016.01.025.

Alvarez-Madrazo, S., Mackenzie, S.M., Davies, E., Fraser, R., Lee, W.K., Brown, M., Connell, J.M., 2013. Common polymorphisms in the CYP11B1 and CYP11B2 genes: evidence for a digenic influence on hypertension. Hypertension. 61 (1), 232–239. https://doi.org/10.1161/hypertensionaha.112.200741.

Amatruda, J.G., Scherzer, R., Rao, V.S., Ivey-Miranda, J.B., Shlipak, M.G., Estrella, M.M., Testani, J.M., 2022. Renin-angiotensin-aldosterone system activation and diuretic response in ambulatory patients with heart failure. Kidney Med. 4 (6), 100465 https://doi.org/10.1016/j.xkme.2022.100465.

Amuthan, A., Chogtu, B., Bairy, K.L., Sudhakar, Prakash, M., 2012. Evaluation of diuretic activity of Amaranthus spinosus Linn. aqueous extract in Wistar rats. J Ethnopharmacol. 140 (2), 424–427. https://doi.org/10.1016/j.jep.2012.01.049.

Bo, Y.Y., Liang, L.D., Hua, Y.J., Zhao, Z., Yao, M.S., Shan, L.B., Liang, C.Z., 2021. High-purity DNA extraction from animal tissue using picking in the TRIzol-based method. Biotechniques. 70 (3), 186–190. https://doi.org/10.2144/btn-2020-0142.

Borges, M., Passos, F.R.S., Quintans, J.S.S., Azeredo, F.J., 2023. Hecogenin and its derivates: a pharmacology review. Biomed Pharmacother. (159), 114251 https://doi.org/10.1016/j.biopha.2023.114251.

Carvalho, Y.M.B.G., Menezes, P.P., Sousa, B.M.H., Lima, B.S., Trindade, I.A.S., Serafini, M.R., Araújo, A.A.S., 2017. Inclusion complex between β-cyclodextrin and hecogenin acetate produces superior analgesic effect in animal models for orofacial pain. Biomed. Pharmacother. 93, 754–762. https://doi.org/10.1016/j.biopha.2017.06.091.

García-Alegría, A.M., Anduro-Corona, I., Pérez-Martínez, C.J., Guadalupe Corella-Madueño, M.A., Rascón-Durán, M.L., Astiazaran-Garcia, H., 2020. Quantification of DNA through the nanodrop spectrophotometer: methodological validation using standard reference material and sprague dawley rat and human DNA. Int J Anal Chem. 2020, 8896738–8896742. https://doi.org/10.1155/2020/8896738.

Huang, X., Cheng, Y., Wang, N., 2022. Genetic variants in CYP11B1 influence the susceptibility to coronary heart disease. BMC Med. Genomics 15 (1), 158. https://doi.org/10.1186/s12920-022-01307-8.

Huber, M., Lezius, S., Reibis, R., Treszl, A., Kujawinska, D., Jakob, S., Kreutz, R., 2015. A single nucleotide polymorphism near the CYP17A1 gene is associated with left ventricular mass in hypertensive patients under pharmacotherapy. Int J Mol Sci. 6 (8), 17456–17468. https://doi.org/10.3390/ijms160817456.

Hullatti, K.K., Sharada, M., Kuppasth, I., 2011. Studies on diuretic activity of three plants from Menispermaceae family. Der Pharmacia Sinica. 2, 129–134.

Ingawale, D., 2020. Saponins and sapogenins of agave with respect to diverse pharmacological role of hecogenin. Int J Pharm Pharm Sci 12 (2), 1–7. https://doi.org/10.22159/ijpps.2020v12i2.35789.

Ingawale, D.K., Patel, S.S., 2016. Anti-inflammatory potential of hecogenin in experimental animals: possible involvement of inflammatory cytokines and myeloperoxidase. Drug Res (Stuttg). 66 (12), 644–656. https://doi.org/10.1055/s-0042-113184.

Jayanthi, M.K., Amoghimath, S., 2018. To evaluate the diuretic activity in ethanolic extract of leaves of delonix regia in wistar albino rats. Biomedical and Pharmacology Journal. 11, 959–963. https://doi.org/10.13005/BPJ/1454.

Lahlou, S., Tahraoui, A., Israili, Z., Lyoussi, B., 2007. Diuretic activity of the aqueous extracts of carum carvi and tanacetum vulgare in normal rats. J Ethnopharmacol. 110 (3), 458–463. https://doi.org/10.1016/j.jep.2006.10.005.

Lee, P.Y., Costumbrado, J., Hsu, C.Y., Kim, Y.H., 2012. Agarose gel electrophoresis for the separation of DNA fragments. J vis Exp. 62, 3923. https://doi.org/10.3791/3923.

Li, X., Ling, Y., Lu, D., Lu, Z., Liu, Y., Chen, H., Gao, X., 2013. Common polymorphism rs11191548 near the CYP17A1 gene is associated with hypertension and systolic blood pressure in the han chinese population. Am. J. Hypertens. 26 (4), 465–472. https://doi.org/10.1093/ajh/hps066.

Liagre, B., Vergne-Salle, P., Leger, D.Y., Beneytout, J.-L., 2007. Inhibition of human rheumatoid arthritis synovial cell survival by hecogenin and tigogenin is associated with increased apoptosis, p38 mitogen-activated protein kinase activity and upregulation of cyclooxygenase-2. Int J Mol Med. 20 (4), 451–460. https://doi.org/10.3892/ijmm.20.4.451.

Liu, Z., Yang, L., Li, L., Wei, R., Luo, X., Xu, T., He, J., 2019. Diuretic and antidiuretic activities of ethanol extract and fractions of lagopsis supina in normal rats. Biomed Res Int., 6927374 https://doi.org/10.1155/2019/6927374.

Melka, A.E., Makonnen, E., Debella, A., Fekadu, N., Geleta, B., 2016. Diuretic activity of the aqueous crude extract and solvent fractions of the leaves of Thymus serrulatus in mice. J Exp Pharmacol. 8, 61–67. https://doi.org/10.2147/jep.s121133.

Novak, J.E., Ellison, D.H., 2022. Diuretics in states of volume overload: core curriculum 2022. Am. J. Kidney Dis. 80 (2), 264–276. https://doi.org/10.1053/j.ajkd.2021.09.029.

Ntchapda, F., Djedouboum, A., Kom, B., Nana, P., Bonabe, C., Maguirgue, K., Dimo, T., 2014. Diuretic activity of the aqueous extract leaves of Ficus glumosa Del. (Moraceae) in rats. ScientificWorldJournal. 2014, 693803 https://doi.org/10.1155/2014/693803.

Pei, R., Duan, C., Vijayalakshmi, A., 2022. Hecogenin attenuates isoproterenol-induced myocardial infarction through nuclear factor-kappa B-mediated signaling pathway in rats. Pharmacognosy Magazine. 18 (78), 463–469. https://doi.org/10.4103/pm.pm_264_21.

Quintans, J.S., Barreto, R.S., de Lucca, W., Villarreal, C.F., Kaneto, C.M., Soares, M.B., Quintans, L.J., 2014. Evidence for the involvement of spinal cord-inhibitory and cytokines-modulatory mechanisms in the anti-hyperalgesic effect of hecogenin acetate, a steroidal sapogenin-acetylated, in mice. Molecules 19 (6), 8303–8316. https://doi.org/10.3390/molecules19068303.

Roush, G.C., Kaur, R., Ernst, M.E., 2013. Diuretics: a review and update. J. Cardiovasc. Pharmacol. Ther. 19 (1), 5–13. https://doi.org/10.1177/1074248413497257.

Santos Cerqueira, G., dos Santos e Silva, G., Rios Vasconcelos, E., Fragoso de Freitas, A. P., Arcanjo Moura, B., Silveira Macedo, D., de Barros Viana, G.S., 2012. Effects of hecogenin and its possible mechanism of action on experimental models of gastric ulcer in mice. Eur J Pharmacol. 683 (1–3), 260–269. https://doi.org/10.1016/j.ejphar.2012.02.043.

Santos Passos, F.R., Pereira, E.W.M., Heimfarth, L., Monteiro, B.S., Gomes, B., de Carvalho, Y.M., Siqueira-Lima, P.S., Quintans, J.S.S., 2021. Role of peripheral and central sensitization in the anti-hyperalgesic effect of hecogenin acetate, an acetylated sapogenin, complexed with β-cyclodextrin: involvement of NFκB and p38 MAPK pathways. Neuropharmacology 186, 108395–108399. https://doi.org/10.1016/j.neuropharm.2020.108395.

Shah, W.A., Jan, A., Khan, M.A., Saeed, M., Rahman, N., Zakiullah, R.A., 2023. Association between Aldosterone Synthase (CYP11B2) gene polymorphism and hypertension in pashtun ethnic population of Khyber Pakhtunkwha, Pakistan. Genes (basel). 14 (6) https://doi.org/10.3390/genes14061184.

Sousa, A.J.C., de Sousa Neto, B.P., da Costa, D.S., de Sousa, M.C., de Carvalho, C.E.S., Quintans-Junior, L.J., de Cássia Meneses Oliveira, R., 2023. Antiulcerogenic and healing activity of hecogenin acetate in rodents. Naunyn Schmiedebergs Arch Pharmacol. 396 (4), 759–769. https://doi.org/10.1007/s00210-022-02341-0.

Titko, T., Perekhoda, L., Drapak, I., Tsapko, Y., 2020. Modern trends in diuretics development. Eur. J. Med. Chem. 208, 112855 https://doi.org/10.1016/j.ejmech.2020.112855.

Van Woudenberg, M., Shin, J., Bernard, M., Syme, C., Abrahamowicz, M., Leonard, G., Pausova, Z., 2015. CYP17A1 and blood pressure reactivity to stress in adolescence. *Int J* Hypertens. 2015, 734586 https://doi.org/10.1155/2015/734586.

Welu, G.G., Yimer, E.M., Hailu, H.G., Bhoumik, D., Lema, M.M., 2020. In Vivo diuretic activity of hydromethanolic extract and solvent fractions of the root bark of clerodendrum myricoides hochst. (Lamiaceae). Evid Based Complement Alternat Med. 2020, 1718708 https://doi.org/10.1155/2020/1718708.

Wróbel, T.M., Jørgensen, F.S., Pandey, A.V., Grudzińska, A., Sharma, K., Yakubu, J., Björkling, F., 2023. Non-steroidal CYP17A1 inhibitors: discovery and assessment. J. Med. Chem. 66 (10), 6542–6566. https://doi.org/10.1021/acs.jmedchem.3c00442.

Yang, L., He, Z.W., He, J.W., 2021. The chemical profiling of aqueous soluble fraction from Lagopsis supina and its diuretic effects via suppression of AQP and RAAS pathways in saline-loaded rats. J Ethnopharmacol. 27, 113951 https://doi.org/10.1016/j.jep.2021.113951.

Zhang, G.X., Wang, B.J., Ouyang, J.Z., Deng, X.Y., Ma, X., Li, H.Z., Zhang, X., 2010. Polymorphisms in CYP11B2 and CYP11B1 genes associated with primary hyperaldosteronism. Hypertens Res. 33 (5), 478–484. https://doi.org/10.1038/hr.2010.21.