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Hesperidin improves physiological outcomes in an arginine vasopressin rat model of pre-eclampsia

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Abstract

Background: Hesperidin, a flavanone commonly found in citrus fruits and herbal formulations, has emerged as a potential new therapeutic agent for modulating several diseases. Since pre-eclampsia is a growing public health threat, it may negatively impact the economy and increase the disease burden of South Africa. Phytocompounds are easily accessible, demonstrate minimal side effects, and may confer novel medicinal options as a treatment and preventive preference.

Objective: To investigate the physiological, biochemical, and hematological outcomes of hesperidin in an arginine vasopressin (AVP)-induced rodent model of pre-eclampsia.

Methods: Female Sprague–Dawley rats were surgically implanted with mini-osmotic pumps to deliver AVP (200 ng/h) subcutaneously. Animals were treated with hesperidin at 200 mg/kg.b.w via oral gavage for 14 days. Systolic and diastolic blood pressures were measured on GD 7, 14, and 18 using a non-invasive tail-cuff method and were euthanized on GD 21.

Results: The findings showed that hesperidin administration significantly decreased blood pressure ($P < 0.05$) and urinary protein levels in pregnant rats ($P < 0.001$). Placental and individual pup weight also increased significantly in the pregnant hesperidin-treated groups compared to AVP untreated groups ($P < 0.001$). Biochemical and hematological markers such as white blood cell count and lymphocyte levels differed significantly ($P < 0.05$) in AVP groups treated with and without hesperidin.

Conclusion: Our results suggest that hesperidin is an antihypertensive agent with modes of action associated with its diuretic and blood pressure lowering effects and reduction of proteinuria in AVP-induced pre-eclamptic rats.

KEYWORDS
blood pressure, hesperidin, natural products, pre-eclampsia, proteinuria

1 | INTRODUCTION

Epidemiological research demonstrates a correlation between a high dietary intake of plant-based polyphenols and a decrease in cardiovascular morbidity and mortality [1–3]. Flavonoids, a group of plant polyphenolic phytochemicals, have been widely explored for

Abbreviations: AVP, arginine vasopressin; BRU, biomedical resource unit; NAVPH, non-pregnant with AVP delivery and hesperidin; NH, non-pregnant with hesperidin delivery; PAVPC, pregnant with AVP delivery and captopril; PAVPH, pregnant with AVP delivery and hesperidin; PAVPS, pregnant with AVP delivery and saline; PH, pregnant with hesperidin delivery; PS, pregnant with saline delivery.

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their pharmacological impact [4–6]. Furthermore, since flavonoids are naturally occurring, they appear to be extremely safe and have no potential for serious side effects, even during pregnancy [7]. One such flavonoid is hesperidin, a plentiful and affordable by-product of citrus farming and the primary phytochemical in oranges, lemons, and other citrus fruits [7]. Hesperidin can account for up to 14% of the fresh weight of young, immature oranges [8]. Multiple studies have demonstrated the health-promoting and pharmacological effects of hesperidin in treating type 2 diabetes, cancer, cardiovascular disease, and neurological and psychiatric disorders [9–11]. Furthermore, hesperidin has exhibited vasodilator, anti-thrombotic, anti-inflammatory, anti-lipemic, and antioxidant activities [12–16], demonstrating its potential use for the treatment and management of a wide range of health conditions. In addition to these, studies have demonstrated the significant anti-hypertensive and diuretic effects of hesperidin in rats [12, 15, 17]. Since hypertension in pregnancy, especially pre-eclampsia, is associated with substantial maternal and fetal morbidity and mortality, access to therapeutic drugs is essential for clinical management [18]. Globally, pre-eclampsia affects 8% of pregnancies [19], with a higher prevalence in low and middle-income countries [20]. This disorder is characterized by new-onset hypertension (≥140/90 mmHg), with/without proteinuria (urinary protein ≥300 mg per 24 h), and multifactorial pathogenesis [21–24]. Furthermore, pre-eclampsia is characterized by modified renal function [25], HELLP (hemolysis, elevated liver functions, low platelet count) syndrome [26], and fetal growth restriction and stillbirth [27].

Current management strategies aim to lower blood pressure and alleviate maternal and neonatal complications [28]. Plant phenolic compounds exhibiting antioxidant, anti-inflammatory, and anti-hypertensive properties may be valuable cost-effective, easily accessible sources for novel pharmaceutical agents in treating pre-eclampsia in low and middle-income countries [29].

Therefore, this study aimed to investigate the effect of hesperidin on physiological, biochemical, and hematological outcomes in an AVP rodent model of pre-eclampsia and establish its applicability in the treatment and management of pre-eclampsia.

2 | MATERIALS AND METHODS

2.1 | Chemicals

AVP (>95%; Mw: 1084.23 g/mol) and hesperidin (80%; Mw: 610.56 g/mol) were purchased from Merck, Germany.

2.2 | Animal welfare

Forty-two pregnant and non-pregnant female Sprague–Dawley rats were acquired from the Biomedical Resource Unit (BRU), Westville Campus, University of KwaZulu-Natal, Durban, South Africa. The animals were maintained under the regulations approved by the Animal Ethics Committee, UKZN (Protocol approval number: AREC/015/020D; date of approval: 26/07/2021). Animals were housed in pairs in medium-sized polycarbonate cages, with a caged area of 646 cm^2 at the BRU under standard laboratory conditions of temperature (22 to 24°C), humidity (60%), and illumination (12-h light/dark cycles), with ad libitum access to standard rat chow and regular drinking water.

2.3 | Experimental study

Of the 42 Sprague–Dawley rats, 24 rats were surgically implanted with ALZET mini-osmotic pumps (model 2004; Durect Corporation, Cupertino, CA) on gestational day (GD) 2 to deliver AVP (200 ng/h) subcutaneously. The study groups ($n = 6$) were defined as follows:

- Group 1: Pregnant with AVP delivery and saline (PAVPS: Negative control)
- Group 2: Pregnant with AVP delivery and hesperidin (PAVPH)
- Group 3: Pregnant with AVP delivery and captopril (PAVPC: Positive control)
- Group 4: Pregnant with saline delivery (PS)
- Group 5: Pregnant with hesperidin delivery (PH)
- Group 6: Non-pregnant with hesperidin delivery (NH)
- Group 7: Non-pregnant with AVP delivery and hesperidin (NAVPH)

The mini osmotic pumps remained implanted until sacrifice. All rats received a dose of hesperidin or captopril (positive control) by oral gavage daily for 14 days from GD 7 to GD 20. The dosage of AVP and captopril was determined based on previous reports [30, 31].

Systolic and diastolic blood pressure were measured on GD 7, 14, and 18 using the MRBP tail-cuff BP monitor (IITC Life Sciences Inc., USA) by placing animals in a suitably sized restrainer. Normal blood pressure was defined as systolic ≤120 mmHg and diastolic ≤80 mmHg. Hypertension in rats was defined as systolic ≥140 mmHg and diastolic ≥90 mmHg. Animals were housed in metabolic cages (Techniplast, Italy) on GD 7, 14, and 18 to collect 24-h urine samples and measure urinary output. Animals were euthanized on GD 21 via isoflurane overdose (Safeline

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Pharmaceuticals, South Africa). Blood samples were collected via cardiac puncture and centrifuged for 15 min at 3500 rpm at 4°C. The number and weight of placentae and pups were recorded. Biochemical and hematological analysis was carried out by a pathology laboratory using rodent reference ranges.

2.4 | Statistical analysis

All statistical analyses were carried out using Stata (Version 12). All data are parametric (SK test) and summarized as mean ± SD. Tukey's post hoc test was used to compare the means between groups. A probability value of $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Effects of hesperidin on systolic and diastolic blood pressure: Non-invasive blood pressure monitoring

Changes in systolic and diastolic blood pressures following the oral administration of saline (negative control), hesperidin, and captopril in all study groups are shown (Figure 1a,b). In all the AVP experimental groups, an initial increase in systolic and diastolic blood pressures (>130–160 and >90–120 mmHg) was observed, followed by a significant decrease in systolic and diastolic blood pressures ($P < 0.05$) after treatment. On GD 18, a significant reduction was noted in both systolic and diastolic blood pressure in hesperidin-

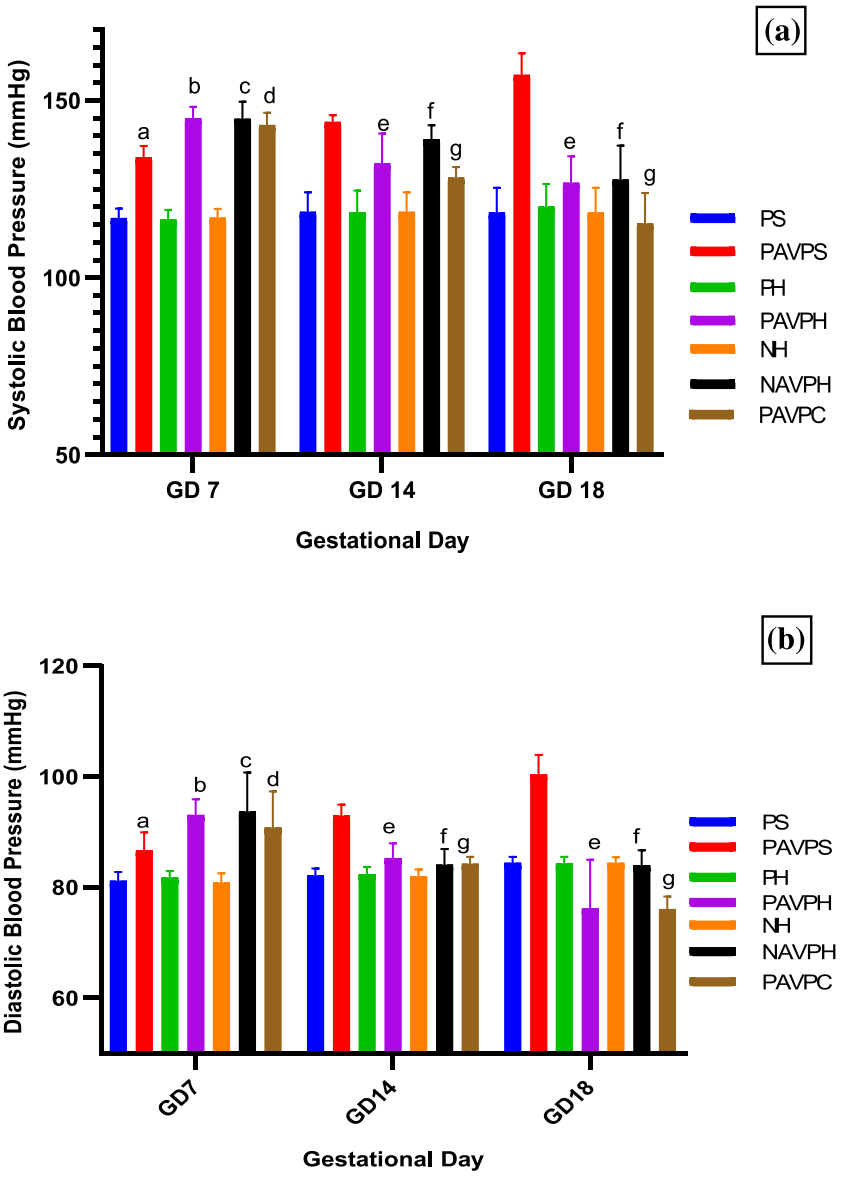


FIGURE 1 Changes in (a) systolic blood pressure and (b) diastolic blood pressure (mean ± SD), observed in study groups on GD 7, 14, and 18, respectively, following oral administration of saline, hesperidin, and captopril. Alphabetic letters ($^{a-g}$) above the bars represent significant differences between groups ($P < 0.05$). ***Key:*** a: PAVPS versus PS, PH, and NH; b: PAVPH versus PS, PH, and NH; c: NAVPH versus PS, PH, and NH; d: PAVPC versus PS, PH, and NH; e: PAVPH versus PAVPS; f: NAVPH versus PAVPS; g: PAVPC versus PAVPS. *Gestational day (GD); pregnant with saline delivery (PS); pregnant with AVP delivery and saline (PAVPS); pregnant with hesperidin delivery (PH); pregnant with AVP delivery and hesperidin (PAVPH); non-pregnant with hesperidin delivery (NH); non-pregnant with AVP delivery and hesperidin (NAVPH); pregnant with AVP delivery and captopril (PAVPC).*

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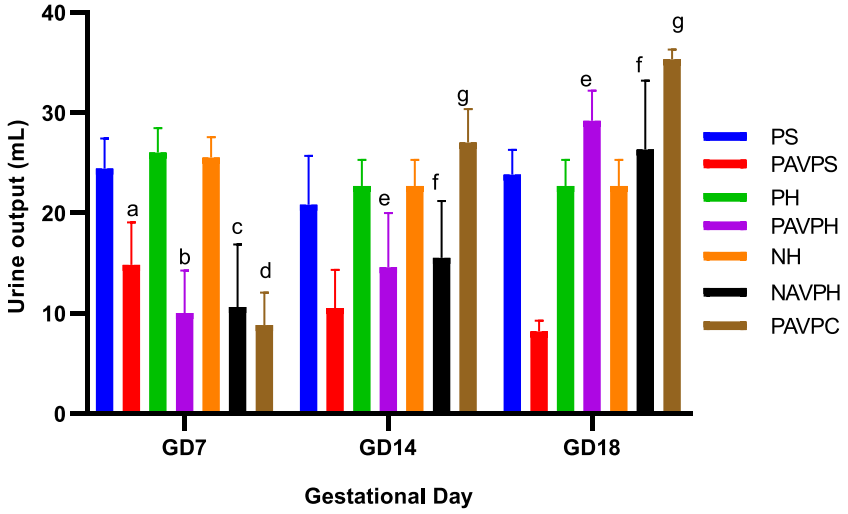


FIGURE 2 Urine output observed in study groups (mean ± SD) on GD 7, 14, and 18, respectively, following oral administration of saline, hesperidin, and captopril. Alphabetic letters ([a–g]) above the bars represent significant differences between groups ($P < 0.05$). ***Key:*** a: PAVPS versus PS, PH, and NH; b: PAVPH versus PS, PH, and NH; c: NAVPH versus PS, PH, and NH; d: PAVPC versus PS, PH, and NH; e: PAVPH versus PAVPS; f: NAVPH versus PAVPS; g: PAVPC versus PAVPS. *Gestational day (GD); pregnant with saline delivery (PS); pregnant with AVP delivery and saline (PAVPS); pregnant with hesperidin delivery (PH); pregnant with AVP delivery and hesperidin (PAVPH); non-pregnant with hesperidin delivery (NH); non-pregnant with AVP delivery and hesperidin (NAVPH); pregnant with AVP delivery and captopril (PAVPC).*

treated animals (PAVPH: 127 ± 7 mmHg; NAVPH: 128 ± 10 mmHg) compared to the negative control group (PAVPS: 157 ± 6 mmHg).

3.2 | Effect of hesperidin on urine volume excretion

Our findings demonstrate a general decrease in urinary volume excretion in the PAVPS group (15 ± 4 mL; 11 ± 4 mL; 8 ± 1 mL) from GD 7 to GD 18 (Figure 2). In contrast, after treatment with hesperidin and captopril, an elevation was noted from GD 14 to GD 18 in the PAVPH, NAVPH, and PAVPC groups (Figure 2).

3.3 | Effects of hesperidin on urine protein and creatinine levels

The urinary protein level of the PAVPS group (2.6 ± 0.3 g/L; $P < 0.001$) was significantly higher than the other experimental groups (Figure 3a). In contrast, a significant reduction in urinary protein levels was noted in the hesperidin and captopril-treated groups compared to the negative control group (PAVPH: 1.0 ± 0.6 g/l; $P < 0.001$; NAVPH: 1.5 ± 0.1 g/L; $P < 0.01$; PAVPC: 1.3 ± 0.2 g/L; $P < 0.001$). Urinary creatinine levels were significantly reduced in the PAVPH (1.7 ± 1.5 mmol/L), NAVPH (2.8 ± 1.2 mmol/L), and PAVPC (0.36 ± 0.1 mmol/L) groups compared to the PAVPS (8.7 ± 1.7 mmol/L) group ($P < 0.001$; Figure 3b). Furthermore, the urinary protein: creatinine ratio significantly ($P < 0.001$) increased in the PAVPS (4.3 ± 1.6 g/mmol) compared to the PS (1.0 ± 0.1 g/mmol) group. A significant ($P < 0.001$) decrease was noted in the PAVPH (0.91 ± 0.8 g/mmol), NAVPH (0.6 ± 0.2 g/mmol), and PAVPC (0.94 ± 0.1 g/mmol) groups compared to PAVPS (Figure 3c).

3.4 | Effects of hesperidin on placental and fetal outcomes

A statistically significant difference ($P < 0.001$; Figure 4a) was noted in the placental weight of the PAVPS (0.45 ± 0.3 g) group compared to PS (0.68 ± 0.5 g), PH (0.71 ± 0.4 g), PAVPH (0.76 ± 0.4 g), and PAVPC (0.67 ± 0.3 g) groups. Similarly, the individual pup weight of the PAVPS (3.5 ± 0.4 g) group was significantly lower than the PS (5.4 ± 0.9 g), PH (5.5 ± 1.0 g), PAVPH (5.9 ± 0.2 g), and PAVPC (6.5 ± 0.1 g) groups, respectively. The number of pups in the PAVPS (9 ± 1) group was significantly lower than in PS (12 ± 1), PH (11 ± 1), PAVPH (14 ± 1), and PAVPC (12 ± 0) groups.

3.5 | Effects of hesperidin on hepatic, renal damage, and electrolyte levels

Table 1 represents the serum biochemical parameters and electrolyte levels across experimental groups on GD 18. Copeptin levels were significantly increased in all AVP-treated experimental groups (PAVPS, PAVPH, NAVPH, and PAVPC; $P < 0.001$; Table 1) versus the non-AVP experimental groups (PS, PH, and NH; $P < 0.001$). The total protein levels were significantly higher in PAVPS compared to PAVPH, NAVPH, and PAVPC groups. A significant reduction was noted in albumin levels for PAVPH versus PAVPS groups.

Aspartate aminotransferase levels were upregulated in the PAVPS compared to PS groups. A significant decrease in aspartate aminotransferase levels was noted in the PAVPH, NAVPH, and PAVPC groups compared to the PAVPS group (Table 1). Furthermore, significantly higher serum alanine transaminase levels were obtained in the PAVPS group compared to the PS ($P < 0.001$) group. In contrast, significantly lower serum

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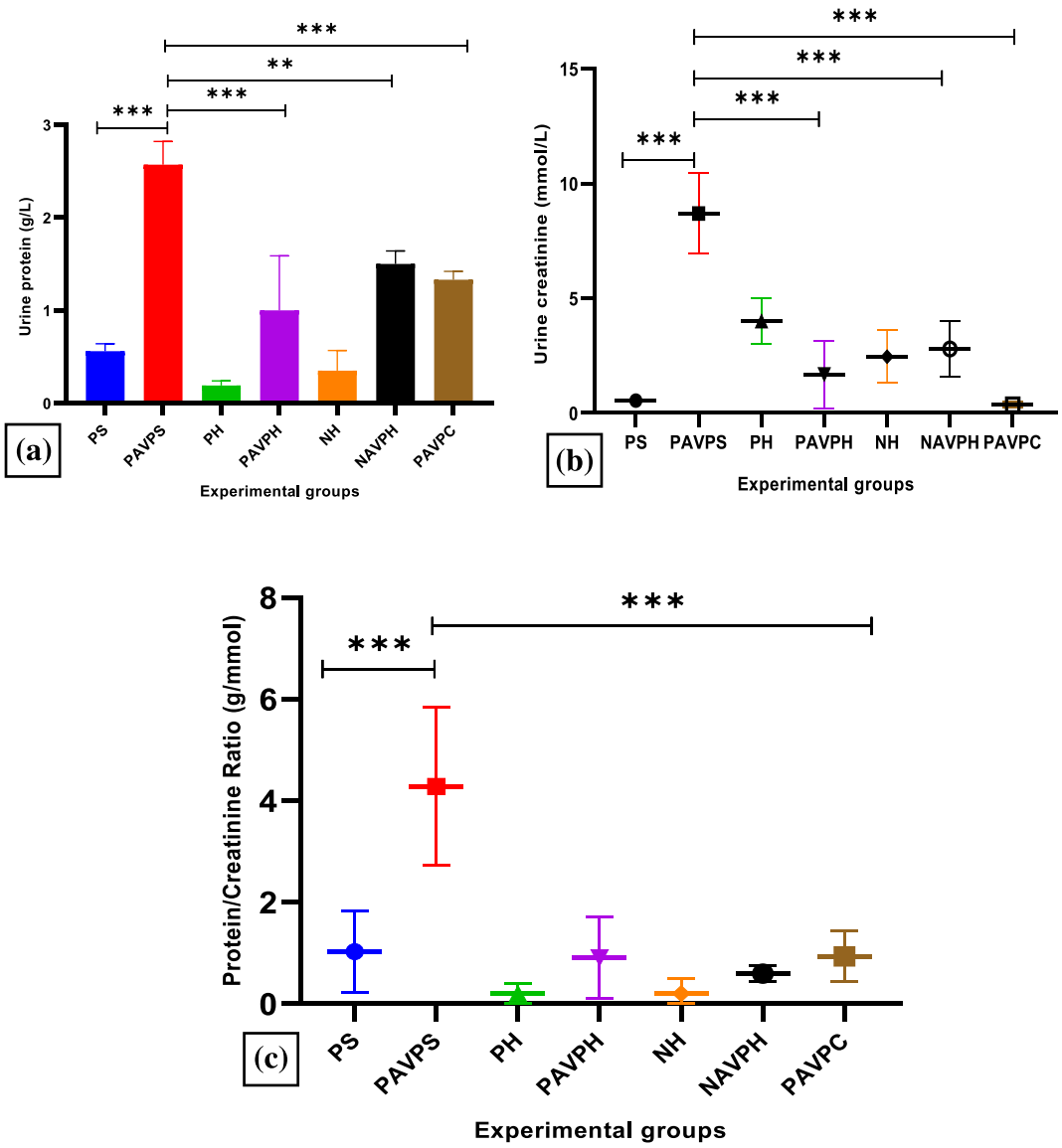


FIGURE 3 Mean ± SD for (a) urinary protein concentration, (b) urine creatinine concentration, and (c) urine protein: creatinine ratio levels across all study groups for GD (18), following oral administration of saline, hesperidin, and captopril $^{**}P < 0.01$, $^{***}P < 0.001$. ***Key**: gestational day (GD); pregnant with saline delivery (PS); pregnant with AVP delivery and saline (PAVPS); pregnant with hesperidin delivery (PH); pregnant with AVP delivery and hesperidin (PAVPH); non-pregnant with hesperidin delivery (NH); non-pregnant with AVP delivery and hesperidin (NAVPH); pregnant with AVP delivery and captopril (PAVPC).*

alanine transaminase levels were observed in the PAVPH, NAVPH, and PAVPC groups than in the PAVPS group (Table 1).

Higher sodium levels were observed in the NH group compared to the PH group. Potassium levels were significantly increased in the NAVPH compared to the PAVPS groups. Chloride levels were significantly lower in the PAVPS compared to PS, NAVPH, and PAVPC groups. However, no significant difference was recorded for the bicarbonate levels between all experimental groups ($P > 0.05$; Table 1).

Urea levels were significantly higher in PAVPS compared to PS groups (Table 1). A decrease in urea levels was also noted in the PAVPH, NAVPH, and PAVPC groups relative to the control. An increase in uric acid levels was seen in the PAVPS compared to PS, PAVPH, and NAVPH groups, albeit, not significant.

3.6 | Effects of hesperidin on hematological parameters

The hematological parameters across all study groups for GD 18 are shown in Table 2. A significantly higher red blood cell count was observed in the PAVPS group compared to the PS and PAVPH groups. Higher red blood cell counts were also observed in the NAVPH group relative to the PS group. A statistically significant difference was also documented for red blood cell counts in the NAVPH compared to PAVPH groups.

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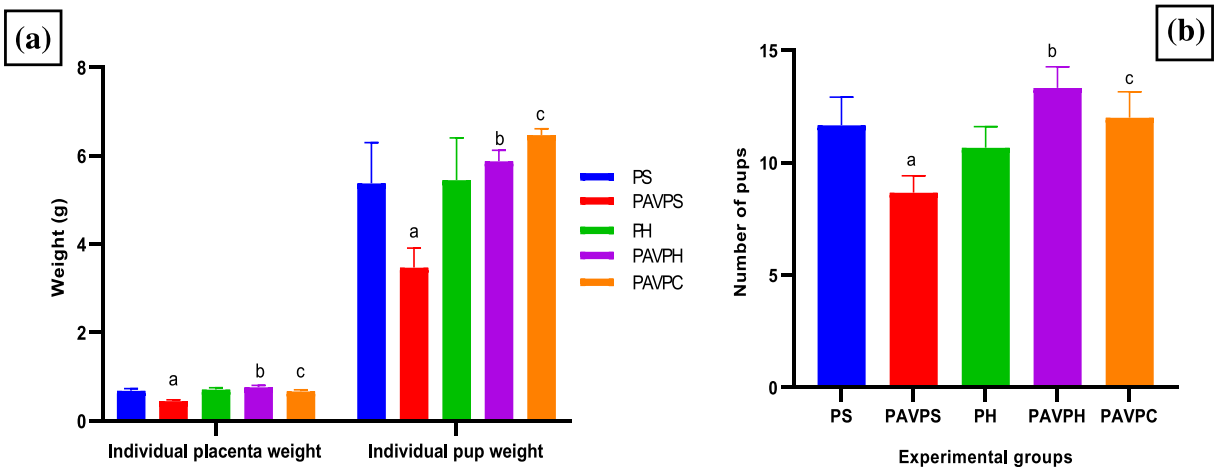


FIGURE 4 Placental and foetal parameters in pregnant Sprague–Dawley rats on GD 18, following oral administration of saline, hesperidin, and captopril (mean ± SD). Alphabetic letters ($^{a–c}$) above the bars represent significant differences between groups ($P < 0.05$). ***Key:*** a: PAVPS versus PS, PH, PAVPH, and PAVPC; b: PAVPH versus PAVPS; c: PAVPC versus PAVPS. *Gestational day (GD); pregnant with saline delivery (PS); pregnant with AVP delivery and saline (PAVPS); pregnant with hesperidin delivery (PH); pregnant with AVP delivery and hesperidin (PAVPH); non-pregnant with hesperidin delivery (NH); non-pregnant with AVP delivery and hesperidin (NAVPH); pregnant with AVP delivery and captopril (PAVPC).*

TABLE 1 Indices of hepatic, renal damage, and electrolyte levels across experimental groups on gestational day (GD) 18.

Study groups								
	PS	**PAVPS**	**PH**	**PAVPH**	**NH**	**NAVPH**	**PAVPC**	***P* value**
Copeptin (pg/mL)	96.74 ± 5.8	653 ± 25.2[a]	129 ± 43.0	557 ± 44.0	134 ± 18.1	427 ± 88.0	494 ± 89.1	0.000
Total protein (g/L)	47.5 ± 1.1[a]	66.3 ± 0.5[cbd]	46.0 ± 2.2	52.3 ± 3.1	43.0 ± 2.9	59.0 ± 1.6	58.5 ± 0.8	0.000
Albumin (g/L)	25.58 ± 2.9	33.40 ± 1.8	33.80 ± 1.66	25.98 ± 1.8[b]	31.45 ± 0.8	31.20 ± 0.4	33.58 ± 3.5	0.011
AST (IU/L)	59.47 ± 4.7[a]	117.33 ± 14.0[e]	60.07 ± 2.07	63.40 ± 9.3[b]	76.55 ± 2.7	90.40 ± 0.6	91.93 ± 8.2	0.000
ALT (IU/L)	46.80 ± 7.8[a]	92.23 ± 5.5[bcd]	55.94 ± 3.95	61.30 ± 12.4	49.48 ± 2.7	57.95 ± 5.6	54.97 ± 4.3	0.000
Sodium (mmol/L)	136 ± 1.8	137 ± 3.5	135 ± 1	138 ± 1.0	142 ± 1.2	142 ± 0.1	139 ± 1.2	0.001
Potassium (mmol/L)	5.23 ± 0.4	4.40 ± 0.2[c]	5.54 ± 0.26	5.20 ± 0.3	5.05 ± 0.2	5.44 ± 0.2	5.47 ± 0.6	0.032
Chloride (mmol/L)	100 ± 2.3	95 ± 2.0[cd]	97 ± 1	99 ± 0.2	100 ± 2.0	101 ± 1.0	101 ± 2.0	0.009
Bicarbonate (mmol/L)	29.47 ± 2.13	28.37 ± 4.1	25.53 ± 2.94	30.35 ± 1.9	31.48 ± 2.2	30.40 ± 1.7	30.85 ± 3.6	0.271
Urea (mmol/L)	4.86 ± 0.1[a]	8.15 ± 0.3[bcd]	5.05 ± 0.61	5.81 ± 0.5	5.02 ± 0.2	6.03 ± 0.02	5.48 ± 0.2	0.000
Uric acid (mmol/L)	0.12 ± 0.3	0.24 ± 0.4	0.14 ± 0.23	0.12 ± 0.4	0.13 ± 0.3	0.17 ± 0.2	0.16 ± 0.2	0.267

Note: Data are shown as mean ± SD. *$P < 0.05$ was considered statistically significant, PS versus PAVPS[a]; PAVPS versus PAVPH[b]; PAVPS versus NAVPH[c]; PAVPS versus PAVPC[d]; NAVPH versus PAVPH[e]. AST—aspartate aminotransferase and ALT—serum alanine transaminase
Key: Pregnant with saline delivery (PS); pregnant with AVP delivery and saline (PAVPS); pregnant with hesperidin delivery (PH); pregnant with AVP delivery and hesperidin (PAVPH); non-pregnant with hesperidin delivery (NH); non-pregnant with AVP delivery and hesperidin (NAVPH); pregnant with AVP delivery and captopril (PAVPC).

Haematocrit levels were significantly higher in PAVPS compared to PS, PAVPH, and PAVPC groups. A considerably higher red cell distribution width was noted in the PAVPS versus NAVPH groups. A lack of statistically significant difference was reported for the mean corpuscular hemoglobin concentration, mean corpuscular volume, and platelets ($P > 0.05$; Table 2).

Our findings also demonstrate significantly lower hemoglobin counts in the PS, PAVPH, NAVPH, and PAVPC groups compared to the PAVPS group. A

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TABLE 2 Haematological parameters across experimental groups on gestational day (GD) 18.

Study groups								
	PS	**PAVPS**	**PH**	**PAVPH**	**NH**	**NAVPH**	**PAVPC**	***P* value**
Red cell count (10^{12}/L)	6.46 ± 0.2[a]	7.99 ± 0.9[b]	6.43 ± 0.2	6.44 ± 0.3[e]	6.39 ± 0.3	8.11 ± 0.7	7.12 ± 0.5	0.000
Haematocrit (%)	41.94 ± 1.7[a]	54.30 ± 2.6[bd]	40.08 ± 1.1	41.52 ± 2.2[e]	44.12 ± 2.2	51.30 ± 4.5	43.50 ± 2.3	0.000
RDW (%)	12.68 ± 0.4	14.76 ± 1.1	12.80 ± 0.3	12.77 ± 0.5[e]	12.03 ± 1.1	12.07 ± 0.6[c]	13.97 ± 1.0	0.000
MCHC (g/dL)	28.56 ± 0.1	27.97 ± 0.4	29.02 ± 0.4	28.50 ± 0.2	28.95 ± 0.9	27.54 ± 2.5	28.52 ± 0.4	0.366
MCV (fL)	63.46 ± 1.8	66.92 ± 8.1	62.36 ± 1.9	64.53 ± 1.6	62.87 ± 2.5	62.29 ± 0.5	62.42 ± 2.5	0.330
Platelets (10^9/L)	1099 ± 94	1011 ± 279	1156 ± 89	1043 ± 123	1074 ± 102	1093 ± 153	1301 ± 244	0.644
Haemoglobin (g/dL)	11.98 ± 0.5[a]	15.85 ± 0.3[cbd]	11.64 ± 0.4	11.83 ± 0.6[e]	13.83 ± 0.8	13.63 ± 1.0	12.40 ± 0.6	0.000
MCH (pg)	17.93 ± 0.2[c]	19.27 ± 0.05[d]	17.80 ± 0.3	18.31 ± 0.4	17.73 ± 0.8	17.87 ± 0.3	17.56 ± 0.8	0.009
White cell count (10^9/L)	4.14 ± 0.5	3.59 ± 0.8[cb]	3.46 ± 0.8	7.64 ± 0.3	4.13 ± 1.0	5.16 ± 0.9	3.69 ± 1.3	0.000
Neutrophils %	28.16 ± 5.3	31.00 ± 4.2[c]	23.80 ± 1.3	29.79 ± 3.2[ce]	8.40 ± 2.3	14.33 ± 4.6	25.38 ± 1.2	0.000
Lymphocytes %	63.78 ± 1.8[a]	59.46 ± 7.8[bc]	62.09 ± 4.2	87.56 ± 2.3	65.20 ± 10.5	74.98 ± 4.3	65.72 ± 4.4	0.000
Monocytes %	3.48 ± 0.9[a]	11.90 ± 0.7[d]	8.50 ± 3.9	6.03 ± 3.7	2.00 ± 0.5	7.81 ± 3.7	4.38 ± 3.0	0.004
Eosinophils %	0.78 ± 0.51	1.57 ± 0.82	1.22 ± 0.41	1.09 ± 0.50	1.53 ± 0.67	1.64 ± 0.65	1.43 ± 0.55	0.303
Basophils %	0.18 ± 0.11	0.18 ± 0.11	0.20 ± 0.11	0.26 ± 0.21	0.37 ± 0.28	0.29 ± 0.15	0.12 ± 0.13	0.392

Note: Data are shown as mean ± SD. **P < 0.05* was considered statistically significant, PS versus PAVPS[a]; PAVPS versus NAVPH[c]; PAVPS versus PAVPH[b]; PAVPS versus PAVPC[d]; NAVPH versus PAVPH. RDW—red cell distribution width; MCHC—mean corpuscular haemoglobin concentration; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin. **Key:** Pregnant with saline delivery (PS); pregnant with AVP delivery and saline (PAVPS); pregnant with hesperidin delivery (PH); pregnant with AVP delivery and hesperidin (PAVPH); non-pregnant with hesperidin delivery (NH); non-pregnant with AVP delivery and hesperidin (NAVPH); pregnant with AVP delivery and captopril (PAVPC).

substantially higher mean corpuscular hemoglobin was noted for the PAVPS group compared to PS and PAVPC groups (Table 2).

Moreover, the white blood cell count was significantly higher in the PAVPH and NAVPH compared to the PAVPS groups. The neutrophil levels were significantly higher in the PAVPS compared to the PAVPH group. A significant decrease in lymphocyte levels was noted in the PAVPS group compared to the PAVPH and NAVPH groups. Similarly, a significant increase was observed in monocyte levels in the PAVPS compared to the PS groups. A lack of statistically significant difference was reported for eosinophils and basophils (*P > 0.05;* Table 2).

4 | DISCUSSION

Our findings demonstrate that hesperidin (200 mg/kg.b.w) administration for 14 days alleviated the AVP-induced increase in blood pressures associated with pre-eclampsia, improving maternal and fetal outcomes. The infusion of AVP (200 ng/h) resulted in significantly elevated blood pressure and urinary protein, characterizing pre-eclampsia symptoms. Both systolic and diastolic blood pressures were elevated considerably throughout pregnancy in the PAVPS group in contrast to the PS group (Figure 1), similar to an earlier report from our lab (Ramdin et al., 2022), albeit at a lower dosage of 150 ng/h. The AVP-induced pre-eclamptic rats treated with hesperidin over 14 days significantly decreased blood pressure. Significant systolic and diastolic blood pressure reductions were reported in the treated groups on gestational days 14 and 18 following treatments with hesperidin. An earlier study reported that treatment with hesperidin for 4 weeks significantly suppressed an age-related increase in blood pressure in spontaneously hypertensive rats [32]. Similarly, spontaneously hypertensive rats administered with hesperidin-rich diets for more than 15 weeks also significantly decreased blood pressure. A more recent study reported that hesperidin reduced blood pressure in a dose-dependent manner and increased plasma ACE activity and angiotensin II levels in two-kidney, one-clipped (2K-1C) hypertensive rats [33].

We report significantly reduced urinary outputs in PAVPS rats compared to the PS rats (Figure 2). The reduced urinary output may be attributed to AVP's antidiuretic effect via V2 receptor activation and increased expression of aqua-porin-2 channels, which results in water retention and consequent decreased urinary output [34]. We further demonstrate a significant increase in urinary production following treatment with hesperidin. Interestingly, our results are corroborated by an earlier study conducted by Galati et al. (1996), who demonstrated significant anti-hypertensive and diuretic

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effects of hesperidin in rats following oral administration of the drug at a dose of 200-mg/kg body weight and ascribed this hypotensive effect to increased diuresis [17].

Although proteinuria is no longer considered a diagnostic indicator of pre-eclampsia [35], it remains an integral diagnostic predictor of the development of pre-eclampsia [36–38]. We demonstrate significantly increased urinary protein and creatinine levels in the PAVPS group, followed by decreased urinary protein and urine creatine levels after treatment with hesperidin (Figure 3a,b). Furthermore, a significantly increased urine protein: creatinine ratio was shown in the PAVPS compared to the PS groups. Interestingly, a significant decrease was noted in the hesperidin AVP-treated groups compared to the PAVPS group (Figure 3c). These findings suggest that hesperidin can ameliorate the progression of pre-eclampsia symptoms and its related kidney dysfunction by reducing proteinuria.

Abnormal placentation in pre-eclampsia leads to reduced placental perfusion, subsequent hypoxia, and fetal growth restriction [39, 40]. We demonstrate significantly lower individual placental and pup weights in the PAVPS versus the PS groups (Figure 4a). Furthermore, the number of pups were markedly lower in the PAVPS compared to the PS groups (Figure 4b). The smaller placentae may be linked to reduced uteroplacental blood flow due to vasoconstriction of spiral arterioles, which directly impacts fetal growth reflecting human pre-eclampsia [41]. We report a significant increase in the weight of the placenta in the hesperidin-treated groups, as well as a significant increase in the pup weight and the number of pups in the hesperidin-treated groups. Our results suggest that hesperidin can be used as a treatment in pre-eclampsia-induced fetal growth restriction, as we reveal significant beneficial effects of fetal weight gain.

Copeptin, a stable protein by-product of AVP synthesis distributed in a 1:1 ratio with AVP, is a biomarker for the measurement of AVP secretion [42]. Our study shows a significant increase in copeptin levels in all AVP-treated groups, and non-significantly lower copeptin levels were noted in the hesperidin-treated groups. AVP stimulates the renin-angiotensin-aldosterone system (RAAS), which causes vasoconstriction, which is mediated by the V1a receptor and, as a result, increases peripheral resistance and systemic blood pressure, as observed in this study [43]. The significant increase in copeptin suggests that hesperidin could act as a vasopressin V1a receptor antagonist, resulting in vasodilation and thereby decreasing blood pressure.

Approximately 3% of pregnancies are affected by liver diseases resulting in maternal and fetal mortality [44]. As a result, liver function tests are critical indicators of liver dysfunction, such as intrahepatic cholestasis of pregnancy or acute fatty liver of pregnancy, which manifests in late pregnancy [44]. These liver injuries are typical in severe cases of pre-eclampsia patients who present with HELLP syndrome [26]. We observed significantly higher serum alanine transaminase and aspartate aminotransferase levels in the PAVPS compared to the PS-treated groups. The depleted serum level of both liver function enzymes in normal and hesperidin-treated groups suggests the protection of hepatocellular injury and the safety of the compound in healthy rats.

In our study, we notice a decrease in the serum concentrations of potassium and chloride in the PAVPS group, which propose a dysregulation in their transport across the vascular smooth-muscle cell membrane [45]. Elevated serum urea and uric acid have been linked with the development of nephropathy [46]. We demonstrate an increase in urea levels in the PAVPS group compared to the treated groups. The increased serum level of urea and acid were ameliorated with the treatment of hesperidin which further supports the ability of hesperidin to abate the progression of pre-eclampsia complications.

We note significantly elevated red blood cell counts and hematocrit levels in the PAVPS group. AVP promotes the proliferation and differentiation of red blood cell precursors, which justifies increased red blood cell counts and hematocrit levels [47]. Elevated hematocrit and red blood cell levels result in an upsurge of blood viscosity and peripheral resistance, leading to increased blood pressure [48].

Recently, red cell distribution width has been associated with hypertension and many other cardiovascular risk factors [49]. Furthermore, high red cell distribution width levels are believed to reflect increased inflammation [49]. We report a higher red cell distribution width percentage in the untreated PAVPS group, which correlates with an earlier study that revealed that red cell distribution width was associated with the presence and severity of pre-eclampsia [49]. Despite the non-significant differences in platelet counts, we note a decreased level in the PAVPS group compared to the other groups. Reduced platelet count is associated with developing HELLP syndrome in severe pre-eclampsia, characterized by hemolysis, elevated liver enzyme, and low platelet count [23]. We note increased hemoglobin and mean corpuscular hemoglobin levels in the untreated control group. An earlier study suggested that an increased free hemoglobin concentration was the cause of vasoconstriction in pre-eclampsia [50]. Moreover, our results are supported by a study which showed that women with high hemoglobin concentration carried an increased risk of pregnancy-induced hypertension [51].

We report higher levels of neutrophils in the untreated PAVPS group. Earlier evidence suggests that neutrophils are activated in the placental bed and maternal circulation of women with pre-eclampsia [52–54]. Furthermore, neutrophil activation is

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associated with a free radical release that either can affect endothelial function directly or contribute indirectly through the production of lipid peroxides [54]. We report elevated white blood cell and lymphocyte levels in the hesperidin-treated groups; however, a significantly lower level in the untreated group. The significant increase in the white blood cell and lymphocytic count caused by hesperidin reflects the compound's leukopoietic and possible immunomodulatory effects [55].

5 | CONCLUSION

These results indicate the ability of hesperidin to improve blood pressure and reduce proteinuria, thus improving maternal and fetal outcomes. The pharmacological action of hesperidin in ameliorating liver dysfunction and improving hematological parameters suggests its promising use in treating pre-eclampsia. However, this plant-derived compound must be effectively studied at the clinical level to establish its usefulness in treating and preventing pre-eclampsia and other hypertensive disorders of pregnancy.

AUTHOR CONTRIBUTIONS

Rebecca Reddy: Experimental work, analysis, and original draft preparation; **Sooraj Baijnath:** Conceptualization, review, and editing; **Sanil Singh**: Methodology; **Roshila Moodley** and **Thajasvarie Naicker**: Review and editing; **Nalini Govender**: Conceptualization, review, and editing.

CONFLICT OF INTEREST STATEMENT

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.

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