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Maternal EGCG intervention mitigates chronic hypertension during pregnancy in spontaneously hypertensive rats without adverse effects on pregnancy outcomes

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ABSTRACT

Background: Chronic hypertension during pregnancy is a significant concern, associated with increased risks of maternal-fetal morbidity and mortality. Epigallocatechin gallate (EGCG), a compound known for its cardioprotective properties, has gained attention as a potential health supplement due to its favorable safety profile.

Objective: This study aims to investigate the effects of maternal EGCG supplementation on elevated blood pressure and pregnancy outcomes in a rodent model of chronic hypertension, specifically using spontaneously hypertensive rats (SHR). Furthermore, the study explores the influence of maternal EGCG supplementation on the blood pressure of SHR offspring during early postnatal development.

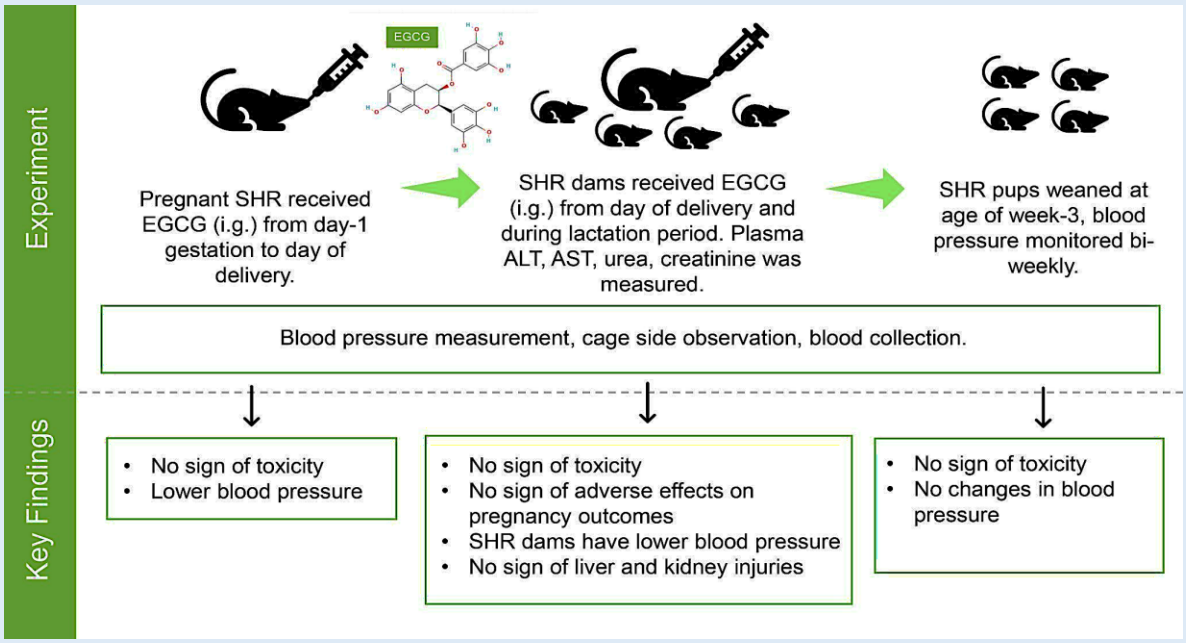
Methods: SHR dams received oral EGCG at 30 mg/kg body weight. Systolic blood pressure (SBP) monitored weekly throughout gestation period and until postpartum day 21. Pregnancy outcomes - litter size, pup viability, and birth weights - were recorded. SBP in weaned SHR offspring was monitored from 5 to 13 weeks of age to assess long-term effects of maternal EGCG treatment. Daily cage-side observations evaluated general health, behavior, and signs of

toxicity. Plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine were analyzed to evaluate liver and kidney function.

Results: EGCG treatment in SHR dams progressively reduced maternal SBP throughout gestation and the postpartum period. However, EGCG administration did not affect pregnancy outcomes (gestation duration, litter size, and birth weights). Markers of liver and kidney function (ALT, AST, urea, and creatinine) showed no signs of organ injury in EGCG-treated groups. Contrary to expectations, SBP in SHR offspring exposed to perinatal EGCG did not decrease compared to control groups, indicating maternal EGCG did not alter the offspring's hypertension predisposition.

Conclusion: Maternal EGCG supplementation effectively lowered blood pressure in hypertensive dams without compromising pregnancy outcomes or causing liver and kidney damage. These findings suggest that EGCG may be a safe cardioprotective supplement during pregnancy. However, perinatal EGCG exposure did not alter the inherent genetic predisposition to hypertension in SHR offspring.

Keywords: Theaceae; *Camellia sinensis*; green tea; catechins, EGCG; hypertension, pregnancy outcomes, perinatal, spontaneously hypertensive rats, chronic hypertension during pregnancy



INTRODUCTION

Chronic hypertension is a major public health concern, affecting 31% of the global population and 7.7% of women of reproductive age. Approximately 6-8% of pregnant women are diagnosed with chronic hypertension, making it one of the most common medical conditions among pregnant women. Hypertension during pregnancy increases the risk of maternal acute renal failure, pulmonary edema, cerebrovascular complications, stillbirth, intrauterine growth restriction, low birth weight, and preterm

delivery [1-3]. Treating hypertension during pregnancy can reduce maternal-fetal health complications.

However, traditional antihypertensive medications pose substantial risks to the fetus during gestation. Angiotensin- converting enzyme inhibitors (e.g., enalapril and lisinopril) and angiotensin II receptor blockers (e.g., losartan and valsartan) may cause low ammonitic fluid levels, fetal renal failure, and birth defect [4-5]. Direct renin inhibitors like aliskiren are generally not recommended during pregnancy due to potential fetal toxicity [6-8]. Thus, options for hypertension management in pregnant women are limited, emphasizing the need for a safe, novel antihypertensive molecule.

Green tea has gained popularity as a functional food due to its nutritional profile and therapeutic benefits, largely attributed to epigallocatechin gallate (EGCG) (Figure 1). Recognized for its antioxidant, anti-inflammatory, and cardiovascular-protective properties, EGCG drives green tea's health appeal and growth in the global market [9-10]. Notably, EGCG has garnered interest as a cardioprotective agent with blood pressure-lowering effects. Our studies demonstrated that EGCG reduces elevated blood pressure by inhibiting the renin-angiotensin-aldosterone system pathway in spontaneously hypertensive rats [11-12]. EGCG also acts as a vasodilator by triggering endothelial nitric oxide production in hypertensive rodent models [12-13]. Clinical studies and meta-analyses, including randomized controlled trials, have concluded that green tea/catechin/EGCG lowers blood pressure in pre-hypertensive and hypertensive individuals [14-17].

Our previous studies indicate that hypertension progression in SHR can be effectively modified through targeted intrauterine and perinatal interventions. Strategies such as perinatal antioxidant administration or maternal environmental manipulation during these critical developmental windows have demonstrated significant potential in mitigating hypertension risk in offspring [18-19]. Prior research has highlighted the beneficial impact of maternal green tea extract/EGCG on mitigating intrauterine growth restriction, dyslipidemia, glucose intolerance, and neural tube defects in offspring [20-22]. Moreover, a randomized clinical trial by Shi et al. (2018) demonstrated that co-administering maternal EGCG improved the effectiveness of oral nifedipine treatment in targeting pregnancy-induced severe pre-eclampsia [23]. However, co-administration of EGCG with nadolol and several cardiovascular drugs raises concerns about potential herb-drug interactions affecting drug pharmacokinetics and bioavailability [24-25].

This present study investigates whether EGCG administration during pregnancy reduces elevated blood pressure in a chronic hypertension model using spontaneously hypertensive rates (SHR). Furthermore, this study hypothesizes that early-life EGCG intervention may reprogram the genetic predisposition to hypertension in SHR offspring, offering a promising research opportunity.

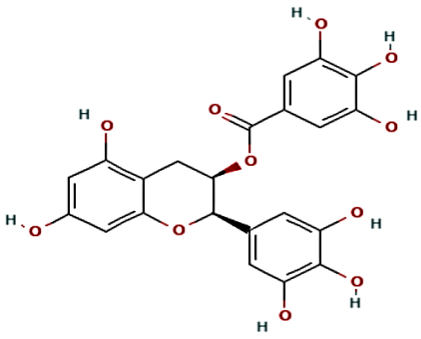


Figure 1: Chemical structure of EGCG (PubChem ID: 65064)

METHODS

Materials and reagents: Crystalline EGCG (Teavigo®) with the purity of > 98% was purchased from Taiyo Kagaku Co., Ltd.

Experimental design: The experimental protocol was approved by the Institutional Ethics Committee (U/SERC/702020). Spontaneously hypertensive rats (SHR) aged 15-16 weeks were obtained from Monash University (Malaysia Campus, Malaysia). The rats were housed under controlled conditions, with temperatures maintained between 22°C and 24°C and a 12-hour light-dark cycle. They had unrestricted access to standard chow and water throughout the study. Baseline systolic blood pressure (SBP) was measured in virgin female and male SHR before cohabitation. Rats with a basal SBP < 160 mmHg were excluded. Vaginal smears from female SHR were collected to determine their estrus cycle stage. Upon confirmation of proestrus, a female SHR was cohabited overnight with a male SHR. Successful mating and pregnancy were confirmed by sperm presence in the vaginal smear the following morning [26,27]. Pregnant SHR were then isolated and randomly divided into two groups: (1) control and (2) EGCG.

EGCG (30 mg/kg body weight/day) was dissolved in phosphate-buffered saline and administered to pregnant SHR via oral gavage from gestation day 1 to postpartum day 21, while controls received PBS. Pregnant SHR were monitored twice daily for delivery. The day of delivery was designated postpartum day 1 for dams and postnatal day 1 for pups. Litter size was culled to 8 pups/litter to minimize milk nutrient intake variation. All pups were weaned at postnatal day 21 and kept EGCG-free thereafter. Male offspring were used for cage-side observation and blood pressure monitoring. SHR dams were sacrificed on postpartum day 22, while offspring were sacrificed at 13 weeks. Blood samples were collected for liver function biomarkers (ALT, AST) and kidney function biomarkers (urea, creatinine). Systolic blood pressure measurement

Systolic blood pressure (SBP) was measured using the indirect plethysmography tail-cuff method (CODA Monitor, Kent Scientific Corporation, Torrington, CT, USA). The SBP of SHR dams was measured on gestation days 1, 14, and 21, and postpartum days 14 and 21. In contrast, SBP measurements for SHR offspring were taken at 5, 7, 9, and 13 weeks of age. All animals underwent training to acclimate to restraint conditions.

Observation of Behavioral Changes & General Toxicity: SHR dams and offspring were monitored twice daily for general health, movement, posture, breathing patterns, eye and skin conditions, piloerection changes, food and water intake, urination, and defecation, as described in our previous study [11].

Measurement of Pregnancy outcomes: Pregnancy outcomes were measured by duration of gestation, number of pups per little (litter size), and birth weight.

Measurement of Liver and Kidney Function Biomarkers: Plasma levels of liver function biomarkers, ALT and AST, were measured using commercially available assay kits (Cayman Chemical, Ann Arbor, MI, USA). Plasma creatinine and urea levels, indicators of kidney function, were determined using the Biolis 24i Premium automated biochemical analyzer (Tokyo, Japan).

Statistical analysis: Results are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA). Continuous

data were evaluated for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). Data confirming normal distribution were compared using an independent samples t-test for two-group comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS

Systolic Blood Pressure in SHR dams and offspring: No significant differences were observed in the basal systolic blood pressure (SBP) of SHR dams prior to oral phosphate-buffered saline (PBS) or epigallocatechin gallate (EGCG) treatment. However, EGCG significantly lowered SBP in SHR dams at gestation days 7, 14, and 21, and postpartum days 14 and 21, compared to the control group receiving PBS (Figure 2, Table 1). This study demonstrates EGCG's effectiveness in reducing SBP in SHR dams.

Cage side observations: EGCG-treated and untreated SHR exhibited comparable activity, alertness, and reaction to handling. Additionally, their respiration patterns, movements, and postures were normal. Ocular examinations revealed clear eyes without any signs of discoloration, discharge, secretions, or inflammation. Their fur was smooth and well-groomed, with skin fully covered and no signs of sores or injury. Furthermore, defecation and urination patterns were normal, and food and water intake were comparable between the EGCG-treated and untreated groups.

Pregnancy Outcomes: No significant differences were found in the gestation period (Figure 4A), litter size (Figure 4B), and birth weight (Figure 4C) between SHR dams that received EGCG and those that did not (Table 1).

Liver and Kidney Function Biomarkers: Regardless of EGCG treatment, there were no significant differences in ALT, AST, urea, and creatinine levels between SHR dams or offspring.

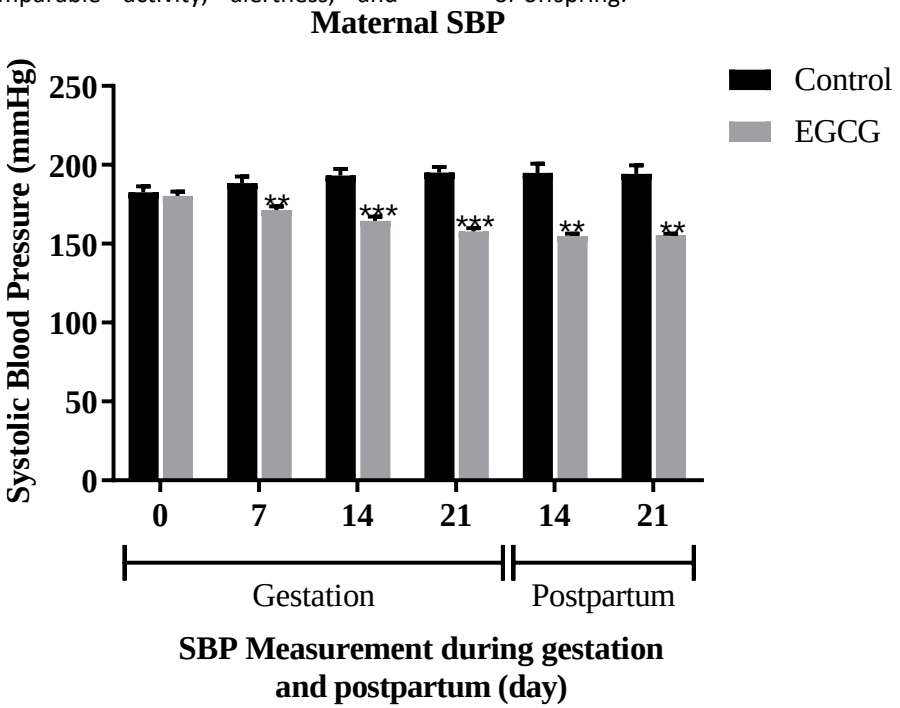


Figure 2: SBP of SHR dams on day-0, day-7, day-14 and day-21 of gestation and day-14 and day-21 of postpartum. **p < 0.01 and ***p < 0.001 *vs.* control group

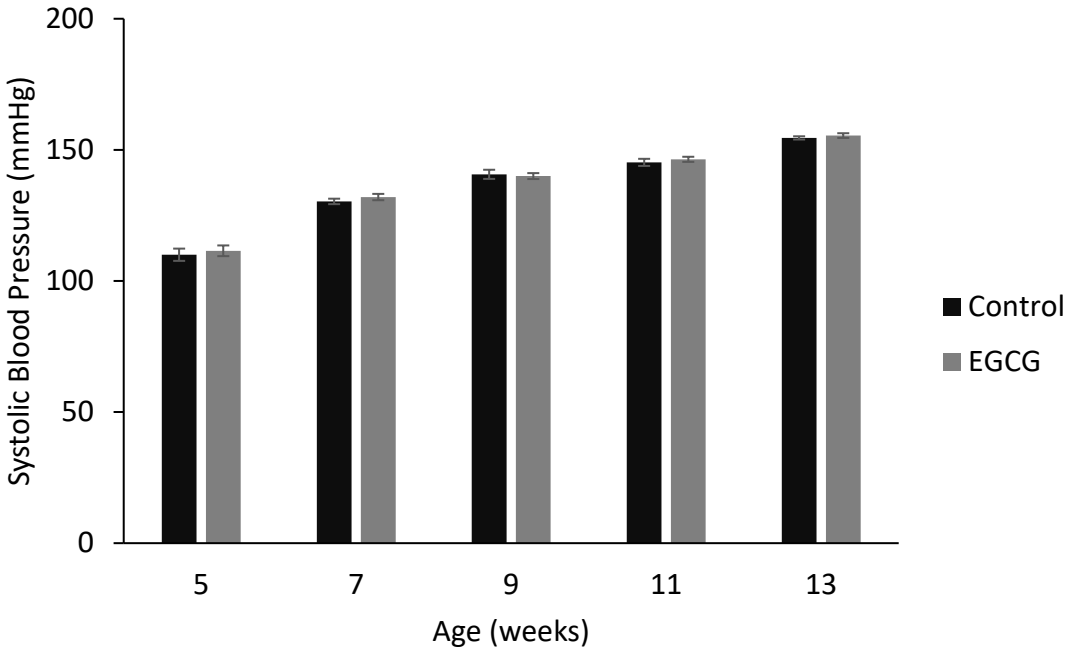


Figure 3: SBP of the male SHR offspring

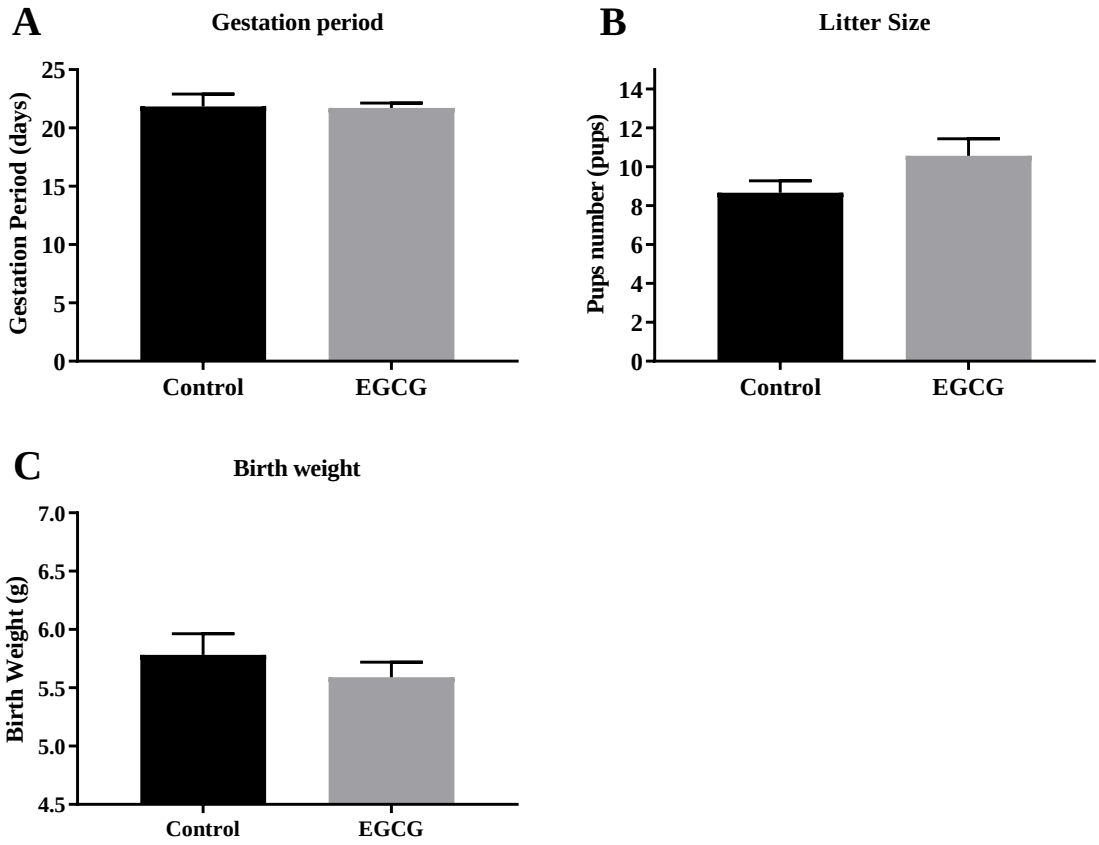


Figure 4: Pregnancy outcomes (A) gestation period (B) litter size and (C) birth weight

Table 1: Pregnancy outcomes, systolic blood pressure of the SHR dams and offspring

Group	Control	EGCG
Gestation period (days)	21.8 ± 1.1	21.7 ± 0.4
Pups number/litter	8.5 ± 0.6	10.3 ± 0.9
Pups Birth weight (g)	5.6 ± 0.2	5.5 ± 0.2
Systolic Blood Pressure (mmHg) during gestation period		
Day-7	188.3 ± 4.3	171.3 ± 2.3***
Day-14	193.2 ± 4.1	164.6 ± 2.7***
Day-21	195.0 ± 3.7	157.7 ± 2.2***
Systolic Blood Pressure (mmHg) during postpartum period		
Day-14	194.8 ± 5.8	154.9 ± 1.5***
Day-21	194.3 ± 5.4	155.6 ± 0.8***
Systolic Blood Pressure of the male offspring at different age		
week-5	109.9 ± 2.3	111.4 ± 2.0
week-7	130.3 ± 1.0	131.9 ± 1.2
week-9	140.6 ± 1.7	140.0 ± 1.1
week-11	145.2 ± 1.3	146.4 ± 1.0
week-13	154.6 ±0.6	155.4 ± 0.9

***p<0.001 *vs.* control

DISCUSSION

The key findings of this study reveal four significant outcomes. Firstly, maternal EGCG administration consistently reduces systolic blood pressure in SHR dams. Secondly, maternal EGCG intake does not adversely affect pregnancy outcomes. Thirdly, EGCG administration exhibits no signs of toxicity in either dams or offspring. Lastly, the blood pressure-lowering effect of maternal EGCG does not persist in offspring.

This study reveals that EGCG's blood pressure-lowering effect is cumulative. The systolic blood pressure reduction in EGCG-treated SHR dams showed a progressive trend, with reductions of 9%, 15%, and 19% observed at gestation days 7, 14, and 21, respectively. Furthermore, the treated dams' systolic blood pressure remained consistently and significantly lower at postpartum days 14 and 21. This sustained reduction highlights EGCG's therapeutic potential in alleviating chronic hypertension during pregnancy.

The absorption and distribution of EGCG in maternal plasma, placenta, and fetus have been well described by Chu et al. [29]. This research confirmed the presence of EGCG in these areas and suggested a potential avenue for in utero protection through maternal EGCG administration. In addition to its potent antioxidant properties, EGCG has been shown to bind to

DNA and RNA molecules, including various transcription factors, thereby modulating gene and protein expression and cellular response [30]. These findings strengthen our research hypothesis, suggesting that maternal EGCG could serve as a potential strategy for early-life intrauterine intervention, potentially reprogramming the genetic predisposition to hypertension in SHR offspring. However, contrary to our initial hypothesis, offspring born to EGCG-treated dams exhibited a non-significant increasing trend in systolic blood pressure (SBP) compared to age-matched counterparts up to 13 weeks of age. SBP measurements were taken 2 weeks after EGCG cessation (offspring were weaned at 3 weeks, and SBP was measured at 5 weeks). Blood pressure measurements were not obtained at weaning due to laboratory equipment limitations. The vanishing blood pressure-lowering effect of maternal EGCG in the offspring remains elusive, possibly attributed to several factors: (i) EGCG might not have effectively traversed the placenta and reached the fetus, (ii) and/or it could be dose-dependent, implying a lack of a lasting impact upon the cessation of treatment.

Gestational period, litter size, and birth weight are crucial indicators of pregnancy outcomes, reflecting the health and development of both mother and fetus. The gestation period refers to the duration of pregnancy, and premature births can lead to various health and developmental issues. In rodents, litter size denotes the number of offspring born in a single pregnancy, with abnormalities potentially indicating maternal health issues or offspring viability concerns. Birth weight reflects intrauterine growth, development, and overall well-being. Previous investigations have established that maternal EGCG administration up to 1000 mg/kg (intragastric) does not adversely affect pregnancy outcomes in normotensive Sprague Dawley rats [31, 32]. However, isolated studies found that prenatal exposure to EGCG at doses as low as 1 mg/kg body weight per day may result in adverse outcomes in adult female C57BL mice, including uterine atrophy, endometrial inflammation, fibrosis [17], and impaired liver function [33]. In contrast, our previous data indicate that EGCG doses of 10-50 mg/kg body weight effectively reduce blood pressure and are generally safe in male spontaneously hypertensive rats (SHR) [12, 24]. This study demonstrates that maternal EGCG administration at 30 mg/kg body weight does not adversely affect pregnancy outcomes in a chronic hypertension model (SHR). Moreover, liver and kidney function markers (ALT, AST, urea, and creatinine) showed no significant changes, supporting EGCG's safety during pregnancy in SHR. These findings align with previous research suggesting that low to moderate doses of EGCG (10-250 mg/kg b.w.) have protective cardiovascular effects without inducing hepatic or renal toxicity [11].

This research pioneers the investigation of EGCG as a therapeutic supplement for managing chronic hypertension during pregnancy, specifically in spontaneously hypertensive rats (SHR), a rodent model genetically predisposed to hypertension. Notably, this study examines the dual impact of maternal EGCG administration, assessing both immediate blood pressure reduction in hypertensive dams and potential long-term cardiovascular effects in offspring – an area previously underexplored. The research provides novel insights into EGCG's safety profile during gestation, highlighting its potential as a safe intervention that reduces maternal hypertension without adversely affecting pregnancy outcomes or causing organ toxicity. Furthermore, by investigating the lack of blood pressure modulation in offspring, this study underscores the limitations of maternal EGCG in altering genetic predispositions to hypertension. This finding offers valuable information for

developing future therapeutic approaches targeting heritable cardiovascular risks.

CONCLUSION

Maternal EGCG supplementation effectively lowered blood pressure in hypertensive dams without compromising pregnancy outcomes or causing liver and kidney damage. These results suggest that EGCG holds promise as a safe cardioprotective supplement during pregnancy. However, perinatal EGCG exposure did not alter the genetic predisposition to hypertension in SHR offspring. These findings provide valuable insights into EGCG's therapeutic potential, highlighting its immediate benefits for maternal cardiovascular health. Furthermore, they pave the way for future research to investigate the underlying mechanisms and explore the long-term effects of EGCG on both maternal and offspring health, including its potential to influence gene-environment interactions and epigenetic modifications in hypertensive conditions.

Abbreviations: Epigallocatechin gallate: EGCG; spontaneously hypertensive rats: SHR; systolic blood pressure: SBP; alanine aminotransferase: ALT; aspartate aminotransferase: AST

Author Contributions: Conception and design of the work Conceptualization, L.S.-K.; data collection; analysis and interpretation of the data, S-Y, L.S.-K, C.A.-L; drafting the manuscript, S-Y, L.S.-K, C.A.-L, C.Y.-L., L.K.B.; critical revision of the manuscript, L.S.-K, C.A.-L, C.Y.-L and L.K.B.

Conflicts of Interest: The authors report no declarations of interest.

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