Effect of Rhodomyrtus tomentosa Hassk. on HIF1α and VEGF expressions on hypertension placental

by Martina Restuati

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Original Article

Effect of *Rhodomyrtus tomentosa* Hassk. on HIF1α and VEGF expressions on hypertension placental

[Efecto de *Rhodomyrtus tomentosa* Hassk. sobre las expresiones de HIF1α y VEGF sobre la hipertensión placentaria]

Putri Cahaya Situmorang[1*], Syafruddin Ilyas[1], Doni Aldo Samuel Siahaan[1], Martina Restuati[2], Endang Ratna Sari[1], Chairunisa Chairunisa[1], Muhammad Faldhy Maliki[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Medan, Indonesia.
*E-mail: putri.cahaya@usu.ac.id

Abstract

Context: HIF1α and VEGF are proteins marker oxidative stress and a decrease in placental growth factor (PlGF). Decreasing of HIF1α and VEGF in rats displayed poor trophoblast differentiation, placental abnormalities, and fetal mortality. *Rhodomyrtus tomentosa* is a flowering plant in the *Myrtaceae* family that has the potential to be a source of health-promoting chemicals.

Aims: To analyze HIF1α and VEGF in serum and hypertension placental tissue after giving *Rhodomyrtus tomentosa* (RHO) leaves extract.

Methods: Six treatments were given to the rats that were identified as being pregnant and pregnant rats with hypertension were given RHO with three doses: (a) normal pregnant rats (control); (b) hypertensive rats; (c) hypertensive rats + 100 mg/kg BW of RHO; (d) hypertensive rats +200 mg/kg BW of RHO; and (e) hypertensive rats + 400 mg/kg BW of RHO and (f) hypertensive rats + nifedipine. Under ketamine anesthesia, pregnant rats were removed on their 20th day of gestation. Immunohistochemistry and ELISA were used to assess HIF1α and VEGF protein expression.

Results: There was a significant difference ($p<0.01$) in the expression of HIF1α and VEGF in the labyrinthine zone and yolk sac of the rat placenta between the normal (C-) and hypertensive (C+) groups. HIF1α and VEGF expression decreased when RHO was administered at doses ranging from 100 to 400 mg/kg BW. However, there was no significant change ($p>0.05$) in VEGF expression in the basal zone of the rat placenta across all groups.

Conclusions: *Rhodomyrtus tomentosa* leaves extract decreases HIF1α and VEGF expressions in serum and repairs the tissue of the placenta's labyrinth, basal, and yolk sacs.

Keywords: basal zone; HIF1α; hypertension; labyrinth zone; plant extract; VEGF; yolk sac.

Resumen

Contexto: HIF1α y VEGF son proteínas marcadoras de estrés oxidativo y disminución del factor de crecimiento placentario (PlGF). La disminución de HIF1α y VEGF en ratas mostró una pobre diferenciación del trofoblasto, anomalías placentarias y mortalidad fetal. *Rhodomyrtus tomentosa* es una planta con flores de la familia *Myrtaceae* que tiene el potencial de ser una fuente de productos químicos que promueven la salud.

Objetivos: Analizar HIF1α y VEGF en suero y tejido placentario hipertenso después de administrar extracto de hojas de *Rhodomyrtus tomentosa* (RHO).

Métodos: Se administraron seis tratamientos a las ratas que se identificaron como preñadas ya las ratas preñadas con hipertensión se les administró RHO con tres dosis: (a) ratas preñadas normales (control); (b) ratas hipertensas; (c) ratas hipertensas + 100 mg/kg de peso corporal de RHO; (d) ratas hipertensas +200 mg/kg de peso corporal de RHO; y (e) ratas hipertensas + 400 mg/kg de peso corporal de RHO y (f) ratas hipertensas + nifedipina. Bajo anestesia con ketamina, las ratas preñadas se extrajeron en su día 20 de gestación. Se usaron inmunohistoquímica y ELISA para evaluar la expresión de proteínas HIF1α y VEGF.

Resultados: Hubo diferencia significativa ($p<0.01$) en la expresión de HIF1α y VEGF en la zona laberíntica y saco vitelino de la placenta de rata entre los grupos normal (C-) e hipertenso (C+). La expresión de HIF1α y VEGF disminuyó cuando se administró RHO en dosis que oscilaron entre 100 y 400 mg/kg de peso corporal. Sin embargo, no hubo cambios significativos ($p>0,05$) en la expresión de VEGF en la zona basal de la placenta de rata en todos los grupos.

Conclusiones: El extracto de hojas de *Rhodomyrtus tomentosa* disminuye las expresiones de HIF1α y VEGF en suero y repara el tejido del laberinto, basal y saco vitelino de la placenta.

Palabras Clave: extracto de plantas; HIF1α; hipertensión; saco vitelino; VEGF; zona basal; zona laberinto.

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AUTHOR INFO
ORCID: 0000-0002-9895-1853 (PCS)
0000-0003-2760-2901 (SI)

INTRODUCTION

Hypertension is a condition in which there is a chronic (long-term) increase in blood pressure, which can cause pain and even death in person. A person is said to have hypertension if their systolic blood pressure exceeds 140 mm Hg and their diastolic blood pressure exceeds 90 mm Hg (Kametas et al., 2022). According to the Ministry of Health, around 31.7% of the population in Indonesia suffers from hypertension. Of this group, only 7.2 out of 31.7% are aware of the condition, and only 0.4% actually use medicine to treat it (Kurnianto et al., 2020). Pregnancy-related hypertension is frequent (6–10%) and raises the risk of morbidity and mortality in mothers, fetuses, and newborns (Kurnianto et al., 2020). Pregnancy-related severe hypertension and pre-eclampsia/eclampsia are more dangerous. Pre-eclampsia/eclampsia, gestational hypertension, chronic pregnancy-related hypertension, and chronic pregnancy-related hypertension with pre-eclampsia are several types of pregnancy-ated hypertension (Braunthal and Brateanu, 2019). Antihypertensive medication used to treat pregnancy-related hypertension had no influence on the likelihood of maternal mortality, proteinuria, side effects, cesarean section, neonatal death, premature birth, or small-for-gestational-age birth (Braunthal and Brateanu, 2019).

An abrupt change in oxygen during pregnancy with hypertension disrupts the placenta's growth (Maria and Warrington, 2019). Pre-eclampsia begins due to an inadequate oxygen supply and ischemia-reoxygenation harm to the defective placenta (Villanueva-Toledo et al., 2020). If the spiral arteries are not properly remodeled during early placentation, there will not be enough nternal blood flow to the placental intervillous area to meet the needs of the fetus for oxygen (Zhang et al., 2021). Intermittent blood flow in the pre-eclamptic placenta causes oxidative stress in the fetomaternal interface syncytiotrophoblast (Zhang et al., 2021). At the heart healthy placentation are these exact adjustments in trophoblast cellular function in response to gestational age-specific alterations in placental oxygenation (Zhang et al., 2021).

Low oxygen levels have a remarkable effect on the induction of HIF protein, and synchronized cytotrophoblasts will result in an increase in HIF1α expression (Siragher and Sferruzzi-Perri, 2021; Strowitzki and Taylor, 2019). HIF1α is a protein highly expressed in various human malignancies, and its expression is frequently associated with a poor prognosis for the patient (Li et al., 2018). HIF1α also causes a drop in placental growth factor synthesis (PlGF). HIF protein is stable and boosts target gene expression in hypoxic/ischemic conditions (Phipps et al., 2019). The HIF protein is crucial for a successful embryo's implantation and placentation, according to earlier research (Li et al., 2018). Rats with HIF1α deletion had poor trophoblast differentiation, placental defects, and fetal mortality (Strowitzki and Taylor, 2019). Impaired placental vasculogenesis is the result of an unchecked rise in HIF protein. These findings imply that the HIF protein is critical for the differentiation of trophoblasts and the control of placental vascular development (Phipps et al., 2019).

Important angiogenesis regulators VEGF and HIF1α are overexpressed in a variety of hypoxic situations (Morfoisse et al., 2014). Pharmaceuticals that target this route include VEGF inhibitors, although one of its major toxicities, hypertension, can have serious negative effects, including the early termination of therapy if optimal blood pressure management cannot be attained (Morfoisse et al., 2014). In most patients, the VEGF receptor signaling pathway causes a rapid but variable increase in blood pressure within three days (Pandey et al., 2018). VEGF is important for blood pressure regulation, and VEGFR-2 is the primary mediator of VEGF's hypotensive effect. Endothelial cells release nitric oxide (NO) and prostacyclin (PGI_2) in response to VEGF, resulting in vasodilation (Robinson et al., 2010). A decrease in microvessel density raises peripheral vascular resistance and decreases NO activity (Pandey et al., 2018).

Orally from Southeast Asia, particularly Indonesia, *Rhodomyrtus tomentosa* Hassk. (haramonting) is a flowering plant of the *Myrtaceae* family (Zhang et al., 2018). This family's distinctive trait is Rhodomyrtone (Vo and Ngo, 2019). There are many advantages to *Rhodomyrtone*, a bioactive acylphloroglucinol molecule that was discovered in the leaves of *R. tomentosa* (Vo and Ngo, 2019). *R. tomentosa* can also reduce HIF1α better in trophoblast cells aged 8 weeks compared to *Zanthoxylum acanthopodium* (Situmorang et al., 2022). These plants can decrease lipid peroxidation, enhance the expression of HSP-70, boost the capacity to scavenge free radicals, and improve the histology of the placenta, testis, and lungs (Ilyas and tumorang, 2021; Ilyas et al., 2019; Irianti et al., 2020; Situmorang and Ilyas, 2018; Situmorang et al., 2020) Due to the high amount of phenolic. It has a strong antioxidant capacity and the potential to be a source of substances that improve health (Vo and Ngo, 2019).

This study med to determine HIF1α and VEGF expression to the herb's potential as a treatment for hypertension in molecular therapy and offer solid justification for its prospective therapeutic application

in contemporary medicine. Before using human cells, it was decided to find out and examine how *R. tomentosa* affected the expression of HIF1α and VEGF in the placental histopathology of hypertensive rats. *R. tomentosa* was reduced in size to a micro colloidal state in order to improve cell penetration and bioavailability. It is envisaged that this plant will be used to create medications for the treatment of hypertension in humans.

MATERIAL AND METHODS

Chemical

This study's key reagents and chemicals included fetal bovine serum (FBS) (Bethesda, MD, USA). 3,3′-diaminobenzidine (Scytek Laboratories, USA) (Scytek Laboratories, USA). Rabbit polyclonal HIF-1alpha IHC antibody (catalog number: IW-PA1041) was used (IHC World, LLC., MD, USA). MA5-12184 VEGF monoclonal antibody (PBS, pH 7.4, with 0.2% BSA) Rat VEGF ELISA kit, catalog #KE20014, ThermoFisher Scientific United States (Proteintech Group, Inc., IL, USA). Catalog#E-EL-R0513, Rat HIFα ELISA Kit (Elabscience, United States).

Vegetal material

R. tomentosa, also known as haramonting, was discovered in Humbang Hasundutan Regency, North Sumatera, Indonesia. The plants were found in the Lintong Nihuta Sub-district of Humbahas Regency, at elevations ranging from 1,000 to 1,500 meters above sea level and located at 02°4'20" - 2°16'15" North latitude and 98°52'40" - 98°56'20" East longitude. Lintong Nihuta District has 479 ha of peatland, accounting for 16.03% of the total peatland area in Humbang Hasundutan Regency (Hutagaol et al., 2021).

Preparation of *R. tomentosa* leaves

The ethanol extract of *R. tomentosa* was created through maceration with a 96% technical ethanol solvent. Five hundred grams of *R. tomentosa* dry powder were macerated with technical ethanol (96%) and tightly closed for 24 hours at room temperature. A Buchner funnel and a vacuum pump were used to filter the maceration results. The filtered residue was macerated twice more using the same method. The *R. tomentosa* ethanol extract was concentrated with a rotary evaporator and dried in a freeze-dryer for eight hours to produce a solid ethanol extract. For sonification, 0.5 mg of *R. tomentosa* extract was added to the Tween 20 solution. Before adding capriol 90 and homogenizing, PEG 400 was added and sonicated. The prepared substance was dissolved in distilled water (1:100) and homogeneously mixed with an ultrasonic device, and the particle size of the clear solution was calculated.

Animal handling

Wistar pregnant female rats who were two months old weighed 150-200 g, were in good health, and were used as the test subjects. The USU Faculty of Mathematics and Natural Sciences Ethics Commission has approved this research as ethical (Ethical Clearance: No. 0302/KEPH-FMIPA/2022). Animals were provided free access to water and fed standard laboratory food in an air-conditioned space with a 12-hour light/dark cycle. Using the CCTV camera built into the cage, a pair of Wistar rats were seen mating during the course of the night. Vaginal plugs on female rats were used to confirm pregnancy, and day 0 was assigned. A tiny osmotic pump was used to introduce *Escherichia coli* lipopolysaccharide (LPS) into the peritoneal cavity, causing systemic inflammation or hypertension in pregnant rats on day 6 of gestation. Animals were ketamine-anesthetized on the 20th day of gestation before being dissected.

Study design

Five treatments were given to the rats that were identified as being pregnant: (a) normal pregnant rats (control); (b) hypertensive rats; (c) hypertensive rats + 100 mg/kg BW *R. tomentosa* (RHO); (d) hypertensive rats + 200 mg/kg BW *R. tomentosa* (RHO); and (e) hypertensive rats + 400 mg/kg BW *R. tomentosa* (RHO); (f) hypertensive rats + 3 mg/kg BW nifedipine (Wang et al., 2019). Under ketamine anesthesia, pregnant rats were removed on their 20th day of gestation. Blood pressure readings were used to identify hypertension markers, and immunohistochemistry was used to assess HIF1α and VEGF protein expression.

ELISA

The blood was drawn from the rats' hearts and allowed to clot at room temperature for 10-20 minutes in an anticoagulant-containing tube. Then, for 20 minutes, centrifuge at a speed of 2000-3000 rpm. The supernatant was then collected and used as a test sample. The ELISA test was used to analyze quantitative HIF-1α and VEGF levels in plasma. Each sample was run three times according to the manufacturer's instructions.

Placental tissue preparation in paraffin and hematoxylin-eosin staining

After being fixed in formalin, the placenta is removed from storage and placed in xylol for 15 minutes. The tissues were then alternatively submerged for five minutes in 96 percent and 70 percent

pure alcohol before being rinsed with distilled water. The tissues were rinsed in distilled water for 3 minutes after being exposed to hematoxylin dye for 5 minutes. Eosin dye is used for 1 minute. Before being submerged in xylol, the slides were dehydrated in 70%, 96%, and 100% alcohol and utilized a light microscope to observe.

Immunohistochemistry of HIF1α and VEGF

To decrease endogenous peroxidase activity, 5 µm thick paraffin-embedded placental slices were deparaffinized and subjected to a 30-minute treatment with 1 percent H_2O_2 in methanol. A 0.01 M Tris-buffered saline wash was then applied to the slides (TBS pH 7.4). Tissue slices were incubated with antigen affinity-purified polyclonal antibody, HIF1α, and VEGF as the antibody utilized in this study and were diluted at 28 g/mL overnight at 4°C. Additionally, immunoreactivity was detected using the Vectastain Elite ABC kit (Vector Laboratories, USA), which was neutralized by Mayer's hematoxylin.

Statistical analysis

The variation was expressed in terms of the standard deviation (SD), along with the number of observations (n). Also, it was declared p-value <0.05, and then the Kruskal-Wallis and Mann-Whitney test were performed for categorical data (ordinal) or numerical data but not regularly distributed (Using SPSS22).

RESULTS

HIF1α analysis after administration of *R. tomentosa*

According to the findings of Table 1, there was a significant difference (p<0.01) in HIF1α expression in the labyrinthine zone of the rat placenta between the normal (C-) and hypertensive (C+) groups. When *R. tomentosa* was administered at doses ranging from 100 to 400 mg/kg, HIF1α expression decreased (p<0.05). Significant differences were also found in the labyrinth of one hypertensive rat that was given the hypertension drug nifedipine (p<0.01). There was also a significant difference (p<0.05) in HIF1α expression between the normal (C-) and hypertensive (C+) groups in the basal zone. However, it was not significant at a dose of 100 mg/kg BW (p>0.05) and significantly different at a dose of 200, 400 mg/kg BW (p<0.05) and nifedipine (p<0.05). Based on the results of the analysis in Table 1, there was a significant difference (p>0.05) in the expression of HIF1α in the yolk sac placenta of rats between normal (C-) and hypertensive (C+) animals. However, it was not significant at a dose of 100 mg/kg BW (p>0.05) and significantly different at a dose of 200, or 400 mg/kg BW (p<0.05) and nifedipine (p<0.05).

The lowest dose produced a positive histological result. In healthy placentas, the HIF1α protein does not appear to be overexpressed in the labyrinthine, basal, or yolk sac zones (Fig. 1A-C). Immunohistochemical analysis of the hypertensive placental labyrinth's basal zone (Fig. 1D-F) revealed a positive result with brown tissue coloration. *R. tomentosa* (RHO) administration resulted in positive outcomes (Fig. 1G-I). As the dose was increased, cell repair began to emerge (Fig. 1J-L). Even at the highest dose, the shape of the placental tissue in the labyrinthine zone resembled that of the control group (Fig. 1M-O). Histological features in the basal zone, labyrinth zone, and yolk sac are almost the same, with the administration of 3 mg/kg BW nifedipine (Fig 1P-R). Image analysis shows that the highest dose of *R. tomentosa* can improve placental histology and reduce HIF1α expression like nifedipine.

Table 1. HIF1α expression placental histology after *R. tomentosa* administration.

Treatment	Labyrinth zone	Basal zone	Yolk sac
C-	14 ± 2.11	10 ± 5.89	10 ± 5.44
C+	42 ± 6.35$^{\#\#}$	22 ± 4.21$^{\#}$	31 ± 4.43$^{\#}$
RHO100	30 ± 8.51*	19 ± 5.22	28 ± 4.91
RHO200	20 ± 4.73*	17 ± 8.04*	20 ± 4.71*
RHO400	18 ± 2.68**	15 ± 7.32*	19 ± 4.53*
NIF	16 ± 3.05**	13 ± 3.12*	17 ± 2.19*

C-: normal pregnant rats (control); C+: hypertensive rats; RHO100: hypertensive rats + 100 mg/kg BW *R. tomentosa*; RHO200: hypertensive rats + 200 mg/kg BW *R. tomentosa*; RHO400: hypertensive rats + 400 mg/kg BW *R. tomentosa*; NIF: hypertensive rats + nifedipine (##p<0.01 *vs.* C-, *p<0.05 *vs.* C+, **p<0.01 *vs.* C+).

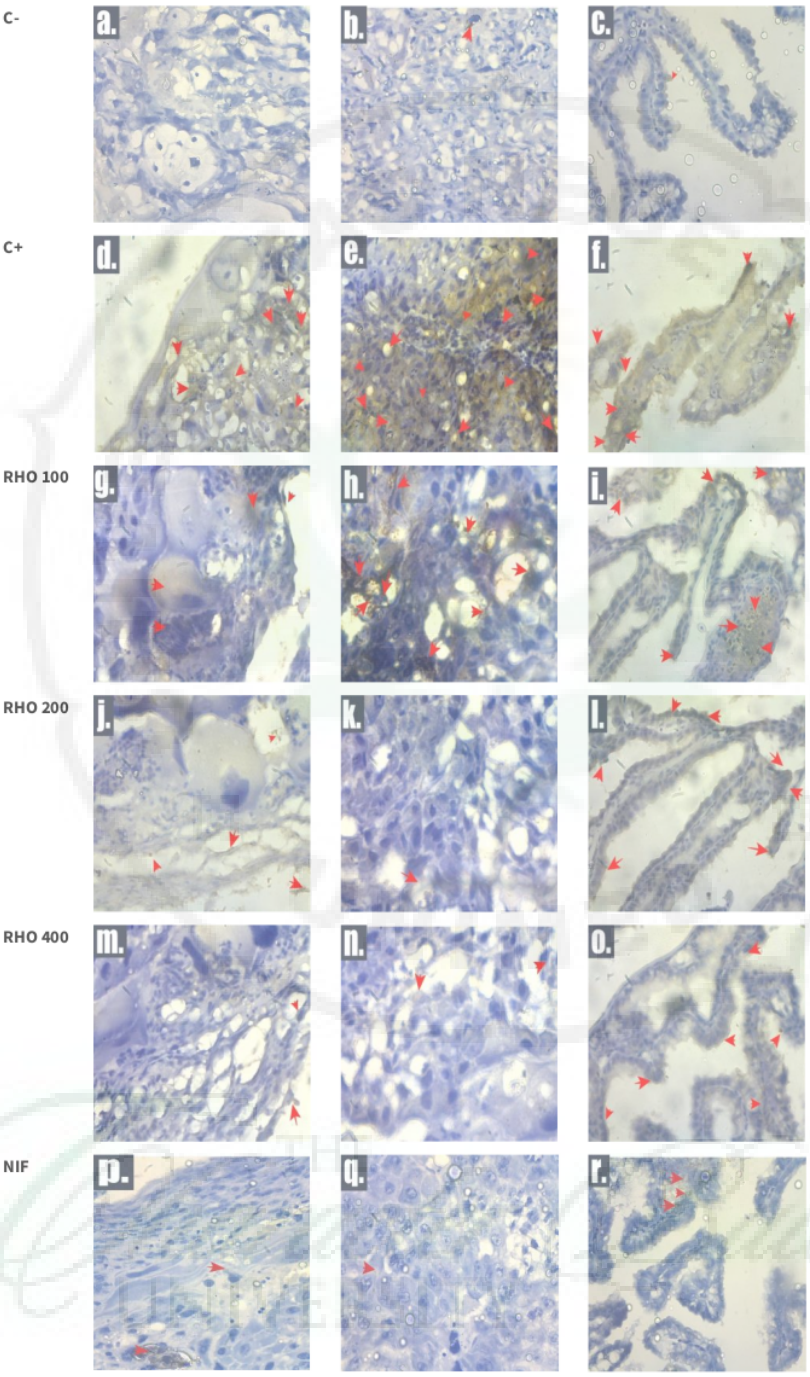

Figure 1. HIF1α expression after administration of *R. tomentosa* on placental histology.

(A, B, C) normal pregnant rats (control); **(D, E, F)** hypertensive rats; **(G, H, I)** hypertensive rats + 100 mg/kg BW *R. tomentosa* (RHO100); **(J, K, L)** hypertensive rats + 200 mg/kg BW *R. tomentosa* (RHO 200); **(M, N, O)** hypertensive rats + 400 mg/kg BW *R. tomentosa* (RHO 400); **(P, Q, R)** hypertensive rats + nifedipine (NIF). Red Arrows: HIF1α expression (40×).

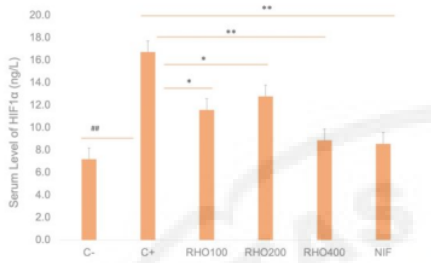

Figure 2. Serum level of HIF1α expression after administration of *R. tomentosa*.

C-: normal pregnant rats (control); C+: hypertensive rats; RHO100: hypertensive rats + 100 mg/kg BW *R. tomentosa*; RHO200: hypertensive rats + 200 mg/kg BW *R. tomentosa;* RHO400: hypertensive rats + 400 mg/kg BW *R. tomentosa* and NIF: hypertensive rats + nifedipine. (##p<0.01 *vs.* C-, *p<0.05 *vs.* C+, **p<0.01 *vs.* C+).

Table 2. VEGF expression placental histology after *R. tomentosa* administration.

Treatment	Labyrinth zone	Basal zone	Yolk sac
C-	8 ± 3.26	8 ± 0.71	9 ± 5.44
C+	20 ± 6.35##	10 ± 4.21	19 ± 4.43#
RHO100	13 ± 8.51*	10 ± 5.22	12 ± 4.91*
RHO200	11± 4.73*	10 ± 8.04	11 ± 4.71*
RHO400	10 ± 2.68**	9 ± 7.32	10 ± 4.53*
NIF	9 ± 1.08**	9 ± 8.61	11 ± 2.43*

C-: normal pregnant rats (control); C+: hypertensive rats; RHO100: hypertensive rats + 100 mg/kg BW *R. tomentosa*; RHO200: hypertensive rats + 200 mg/kg BW *R. tomentosa;* RHO400: hypertensive rats + 400 mg/kg BW *R. tomentosa;* NIF: hypertensive rats + nifedipine. (##p<0.01 *vs.* C-, *p<0.05 *vs.* C+, **p<0.01 *vs.* C+).

The blood serum analysis also revealed that there was a significant difference (p<0.01) in HIF1α expression between the normal group (C-) and the hypertension group (C+). HIF1α expression decreased when *R. tomentosa* was administered at doses ranging from 100 mg/kg to 400 mg/kg (p<0.05). The highest dose has the same statistical value as the administration of highest dose has the same statistical value as the administration of nifedipine p<0.01). The maximum dose can reduce the expression of HIF1α the best compared to the previous dose (Fig. 2).

VEGF analysis after administration of *R. tomentosa*

According to the findings of Table 2, there was a significant difference (p<0.01) in VEGF expression in the labyrinthine zone of the rat placenta between the normal (C-) and hypertensive (C+) groups. When *R. tomentosa* was administered at doses ranging from 100 to 400 mg/kg BW, VEGF expression decreased (p<0.05) and also the nifedipine group (p>0.01). However, VEGF was not expressed in the basal zone in any of the experimental groups (p>0.05). In the yolk sac section, there was a significant difference (p<0.05) in VEGF expression between normal (C-) and hypertensive (C+) animals. Table 2 also describes that there was a significant difference (p<0.05) in all groups given *R. tomentosa* at a dose of 100-400 mg/kg BW and nifedipine in the yolk sac (p<0.05).

In histological analysis, the lowest dose of *R. tomentosa* produced positive results. In the labyrinthine, basal, and yolk sac zones of normal placentas, the VEGF protein does not appear to be overexpressed (Figs 3A-C). Immunohistochemical analysis from the basal zone, placental labyrinth, and yolk sac (Fig. 3D-F) revealed VEGF positivity with brown tissue coloration. Even at the lowest dose, *R. tomentosa* can repair beneficial placental tissue (Fig. 3G-I). As the dose was increased, placental tissue repair improved (Fig. 3J-L). Even at the highest dose, the shape of placental tissue in the labyrinthine zone, basal zone, and yolk sac was nearly identical to that of the control group (Fig. 3M-O). The administration of nifedipine as a drug to treat hypertension and chest pain (angina pectoris) showed the same histological picture in the placenta as the highest dose and control groups and a marked decrease in VEGF expression (Fig. 3Q-R).

The blood serum analysis also revealed a significant difference in VEGF expression (p<0.01) between the normal (C-) and hypertensive (C+) groups. When *R. tomentosa* was administered at doses ranging from 100 to 400 mg/kg BW, VEGF expression decreased (p<0.05) and also the administration of nifedipine (p<0.05). A single dose of *R. tomentosa* can reduce VEGF expression in hypertensive rats' blood serum (Fig. 4).

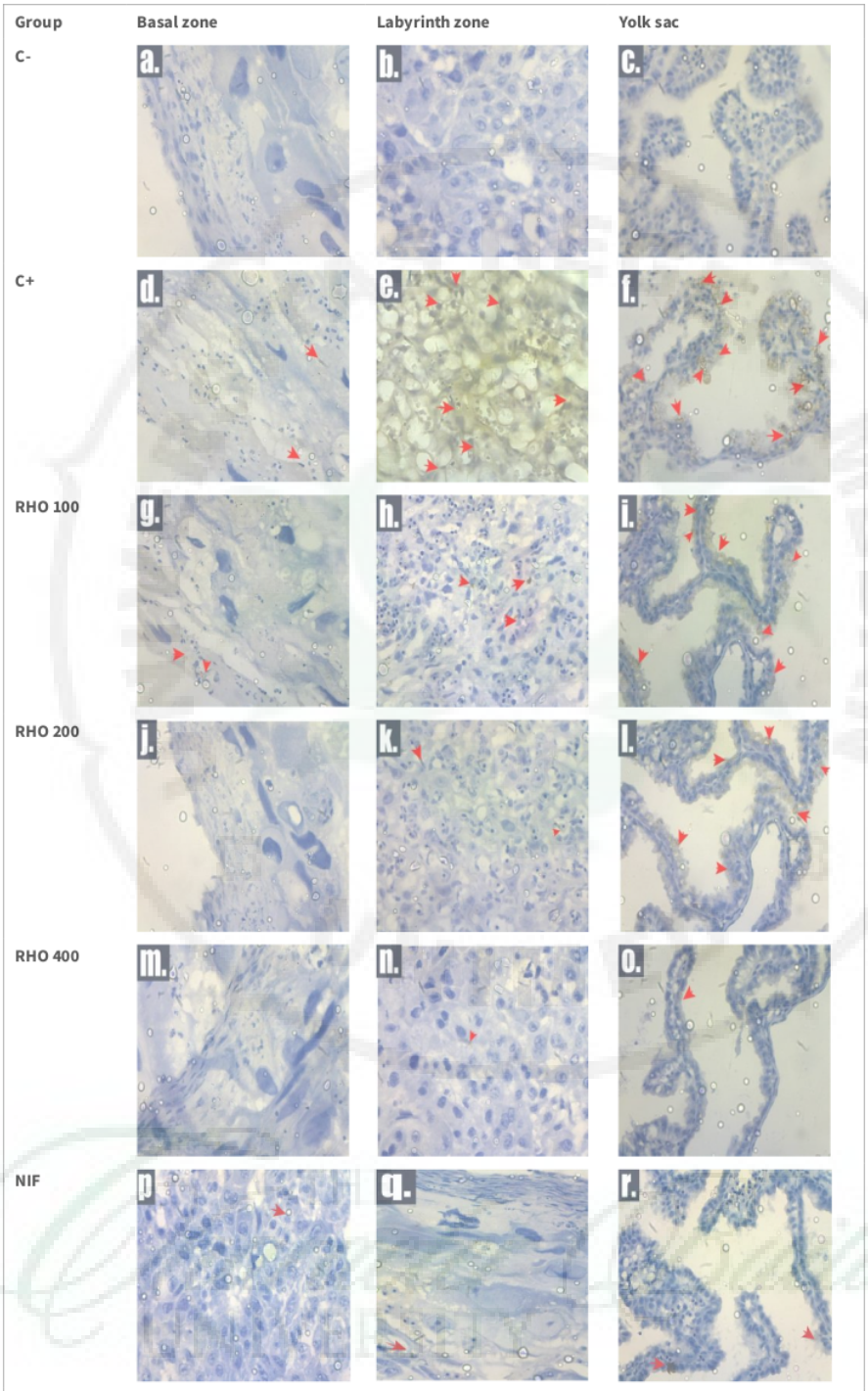

Figure 3. VEGF expression after administration of *R. tomentosa* on placental histology.

(A, B, C) normal pregnant rats (control); **(D, E, F)** hypertensive rats; **(G, H, I)** hypertensive rats + 100 mg/kg BW *R. tomentosa* (RHO 100); **(J, K, L)** hypertensive rats + 200 mg/kg BW *R. tomentosa* (RHO 200); and **(M, N, O)** hypertensive rats + 400 mg/kg BW *R. tomentosa* (RHO 400); (P,Q,R) hypertensive rats + nifedipine (NIF). Red Arrows: VEGF expression (40×).

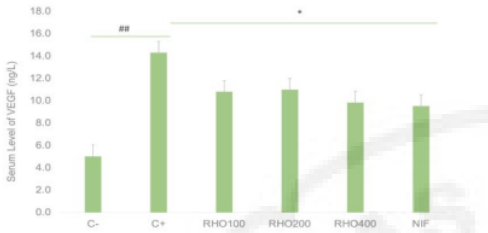

Figure 4. Serum level of VEGF expression after administration of *R. tomentosa*.

C-: normal pregnant rats (control); C+: hypertensive rats; RHO100: hypertensive rats + 100mg/kg BW *R. tomentosa*; RHO200: hypertensive rats + 200 mg/kg BW *R. tomentosa*; RHO400: hypertensive rats + 400 mg/kg BW *R. tomentosa*; and NIF: hypertensive rats + nifedipine. (##p<0.01 *vs.* C-, *p<0.05 *vs.* C+).

DISCUSSION

HIF regulates numerous important aspects of tumor development, growth, invasion, inflammatory cell recruitment, metastasis, and hypertension (Reshef, 2012). As a result, they are appealing targets for future targeted therapeutics. Antioxidants can neutralize free radicals that might harm the body, including the placenta (Salles et al., 2012). There is a split of antioxidants based on the sources obtained, particularly antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which are known as endogenous antioxidants (Salles et al., 2012). Exogenous antioxidants, such as *R. tomentosa*, are derived from outside the body (Salles et al., 2012). This study's oxidant was an antioxidant derived from the leaves of *R. tomentosa*. This plant's extracts contain acylphloroglucinol, flavonoids, tannins, and triterpenes (Vo and Ngo, 2019).

Rats' placentas are both hemotrichorial and discoid (Fukurawa et al., 2019). Histologically, three layers of trophoblast separate the maternal blood space from the fetal blood vessels in the labyrinth zone, notably the outer trophectoderm, which is in direct contact with maternal blood with the surface of the microvilli and is generally referred to as the cytotrophoblat (Fukurawa et al., 2014). The trophectoderm is the second layer, which is made up of two layers of syncytiotrophoblast (Fukurawa et al., 2014). *R. tomentosa* (RHO) was delivered, it demonstrated a decrease in HIF1α expression even at the maximum dose, according to the results of the immunohistochemistry investigation of the hypertensive placental labyrinth (Zhang et al., 2018). The highest dose of *R. tomentosa* can improve placental histology and reduce HIF1α expression like nifedipine. This is due to the presence of antioxidants in *R. tomentosa*, such as anthocyanins, acylphloroglucinol, flavonoids, tannins, and triterpenes, which protect cells against hypoxia and death. In the basal zone, *R. tomentosa* produced a negative result (Vo and Ngo, 2019). The basal zone is divided into three distinct types: spongiotrophoblasts, trophoblast large cells, and glycogen cells. Spongiotrophoblast can be found right above the large cell trophoblast layer at the maternal-fetal placental contact (Malnou et al., 2019; Situmorang et al., 2021). The glycogen cells then divide into multiple tiny cell masses and grow into glycogen cell islands in midpregnancy, with some disappearing before the mother gives birth to the fetus (Malnou et al., 2019). HIF1α expression in hypertension and negative RHO administration in the basal zone shows that the placental interface's maternal-fetal transfer system is not compromised. Hypertension increases HIF1α expression; however, administration of *R. tomentosa* decreases HIF1α expression, and when the dose is increased to the greatest dose, the tissue shape is the same as in healthy placenta (Reshef, 2012). The yolk sac is a component of the fetus that provides sustenance to the fetus in order for it to produce the placenta (Ross and Boroviak, 2020). Yolk sacs have been generated in humans since the gestational age of four weeks. The yolk sac is a membranous sac connected to the embryo and composed of hypoblast cells (Ross and Boroviak, 2020).

VEGF regulates placental vascular function effectively. Endothelial dysfunction is a major risk factor for pre-eclampsia (Opichka et al., 2021). By sequestering excess maternal VEGF, sFLT1 plays an important role in maintaining vascular integrity in the placenta (Fan et al., 2014). This suggests that local increases in VEGF may trigger placental overexpression of sFLT1, potentially contributing to the development of pre-eclampsia and other pregnancy complications (Fan et al., 2014). In the hypertensive group, VEGF immunoreactivity was found in all placental components. In the labyrinth zone, which aids in the exchange of O_2/CO_2, the provision of nutrients to the fetus, and the removal of waste products. Damage to the labyrinth zone is strongly linked to intrauterine growth retardation (IUGR) (Belkacemi et al., 2011; Hemberger, 2012). Because of its high blood flow, high cellular proliferative activity, and long proliferative period compared to other parts of the placenta, the labyrinthine zone is vulnerable to target cells in placental free radicals (Sarkar et al., 2016). The basal zone, which forms just beneath the labyrinthine zone, is made up of spongiotrophoblasts, glycogen cells, and

trophoblast giant cells (secondary). In rats, hypoxia expression increased cell glycogen in the basal zone (Furukawa et al., 2019). The abnormal retention of a large cytoplasmic vacuum in glycogen cells is referred to as cystic degeneration. This is due to the remnants of glycogen cell islands, which should regress and disappear at the end of pregnancy as the placenta develops (Furukawa et al., 2019). When compared to other parts of the placenta, the presence of chemically induced lesions or oxidative stress was significantly different in the basal zone depending on the period of chemical administration (Furukawa et al., 2014). In the fetus, the yolk sac also serves as an endocrine, metabolic, immunological, secretory, excretory, and hematopoietic system (Ross and Boroviak, 2020). Until the formation of the chorioallantoic placenta, it is the only major transport route between the mother and the embryo. As a result of oxidative stress, impaired structural and functional development of the yolk sac contributes to toxicity and teratogenesis in rat embryos (Ross and Boroviak, 2020).

When compared to the control group, VEGF positive expression in all pathological groups showed a higher intensity of reactivity. The different expressions of VEGF in tissues in the placenta may be related to the hemodynamic changes that occur in this disorder in the context of normal uteroplacental blood flow restoration (Fan et al., 2021). When compared to normal placentas, VEGFR-1 protein expression was higher in pre-eclampsia (PE) placentas. Similarly, the mRNA levels of the three VEGF isoforms tested increased, while the protein levels of the two main VEGF isoforms detected did not change in PE placentas compared to normal placental (Fan et al., 2021).

A dose of 100 mg/kg BW resulted in a significant reduction in VEGF expression. Histological features were also similar to those given by the group given nifedipine as a comparison. Nifedipine has been demonstrated to be useful and generally well tolerated for the treatment of stable, variable, and unstable angina, mild to severe hypertension and Raynaud's phenomenon (Wang et al., 2019). It is an antagonist of calcium influx through slow cell membrane channels. The powerful coronary and peripheral arterial dilator capabilities of nifedipine, with enhanced oxygen supply demand and lower systemic vascular resistance, are of tremendous importance, even though the particular mechanism of action in these disorders has not yet been fully understood (Kubo et al., 1981). Both *in vitro* and *in vivo* studies show that *R. tomentosa* extract is a powerful antioxidant. *R. tomentosa is* also a good source of phenolics, with more than 19 individual phenolics identified, including anthocyanins, phenolic acids, flavonols, ellagitannins, and stilbenes (Vo and Ngo, 2019). This herbal leaf's antioxidant content is thought to be responsible for the decreased expression of VEGF in the histology of the placenta and blood serum in hypertensive rats. As a result, *R. tomentosa* can be used as an alternative antioxidant therapy in the pharmacology industry to prevent biomolecule damage caused by free radicals found in a variety of human diseases.

CONCLUSION

The findings reveal that hypertension decreases placental efficiency and increases HIF1α and VEGF expression in rats, and that *Rhodomyrtus tomentosa* treatment reduces HIF1α and VEGF expression from the serum and repairs the tissue of the placenta's labyrinth, basal, and yolk sacs.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Belkacemi L, Desai M, Nelson DM, Ross MG (2011) Altered mitochondrial apoptotic pathway in placentas from undernourished rat gestations. Am J Physiol Regul Integr Comp Physiol 301(6): R1599-R1615. https://doi.org/10.1152/ajpregu.00100.2011

Braunthal S, Brateanu A (2019) Hypertension in pregnancy: Pathophysiology and treatment. SAGE Open Med 7: 2050312119843700. https://doi.org/10.1177/2050312119843700

Fan X, Rai A, Kambham N, Sung JF, Singh N, Petitt M, Dhal S, Agrawal R, Sutton RE, Druzin ML, Gambhir SS, Ambati BK, Cross JC, Nayak NR (2014) Endometrial VEGF induces placental sFLT1 and leads to pregnancy complications. J Clin Invest 124(11): 4941-4952. https://doi.org/10.1172/JCI76864

Fan X, Muruganandan S, Shallie PD, Dhal S, Petitt M, Nayak NR (2021) VEGF maintains maternal vascular space homeostasis in the mouse placenta through modulation of trophoblast giant cell functions. Biomolecules 11(7): 1062. https://doi.org/10.3390/biom11071062

Furukawa S, Tsuji N, Sugiyama A (2019) Morphology and physiology of rat placenta for toxicological evaluation. J Toxicol Pathol 32(1): 1–17. https://doi.org/10.1293/tox.2018-0042

Furukawa S, Kuroda Y, Sugiyama A (2014) A comparison of the histological structure of the placenta in experimental animals. J Toxicol Pathol 27(1): 11–18. https://doi.org/10.1293/tox.2013-0060

Hemberger M (2012) Health during pregnancy and beyond: Fetal trophoblast cells as chief co-ordinators of intrauterine growth and reproductive success. Ann Med 44: 325–337. https://doi.org/10.3109/07853890.2012.663930

Hutagaol JM, Bintang, Hidayat B (2021) Identification of highland peat vegetation in the sub-district of Lintong Nihuta, Humbang Hasundutan Regency, North Sumatera, Indonesia.

IOP Conf Series: Earth Environ Sci 912: 012027 https://doi.org/10.1088/1755-1315/912/1/012027

Ilyas S, Murdela F, Hutahaean S, Situmorang PC (2019) The effect of haramounting leaf ethanol extract (*Rhodomyrtus tomentosa* (Aiton) Hassk.) on the number of leukocyte type and histology of mice pulmo (*Mus musculus* L.) exposed to electronic cigarette. Open Access Maced J Med Sci 7(11): 1750-1756. https://doi.org/10.3889/oamjms.2019.467

Ilyas S, Situmorang PC (2021) Role of heat shock protein 70 (HSP-70) after giving nanoherbal haramonting (*Rhodomyrtus tomentosa*) in preeclamptic rats. Pak J Biol Sci 24: 139-145. https://doi.org/10.3923/pjbs.2021.139.145

Irianti E, Ilyas S, Hutahaean S, Rosidah R, Situmorang PC (2020) Placental histological on preeclamptic rats (*Rattus norvegicus*) after administration of nanoherbal haramonting (*Rhodomyrtus tomentosa*). Res J Pharm Technol 13(8): 3879-3882. https://doi.org/10.5958/0974-360X.2020.00686.1

Kametas NA, Nzelu D, Nicolaides KH (2022) Chronic hypertension and superimposed preeclampsia: Screening and diagnosis. Am J Obstet Gynecol 226(2S): S1182-S1195. https://doi.org/10.1016/j.ajog.2020.11.029

Kubo T, Fujie K, Yamashita M, Misu Y (1981) Antihypertensive effects of nifedipine on conscious normotensive and hypertensive rats. J Pharmacobiodyn 4(4): 294-300. https://doi.org/10.1248/bpb1978.4.294

Kurnianto A, Kurniadi Sunjaya D, Ruluwedrata Rinawan F, Hilmanto D (2020) Prevalence of hypertension and its associated factors among Indonesian adolescents. Int J Hypertens 2020: 4262034. https://doi.org/10.1155/2020/4262034

Li Q, Yao B, Endler L, Chen L, Shibasaki F, Cheng H (2018) Int6/eIF3e silencing promotes placenta angiogenesis in a rat model of pre-eclampsia. Sci Rep 8(1): 8944. https://doi.org/10.1038/s41598-018-27296-2

Malnou EC, Umlauf D, Mouysset M, Cavaillé J (2019) Imprinted microRNA gene clusters in the evolution, development, and functions of mammalian placenta. Front Genet 9: 706. https://doi.org/10.3389/fgene.2018.00706

Maria JM, Warrington JP (2019) Cerebral blood flow regulation in pregnancy, hypertension, and hypertensive disorders of pregnancy. Brain Sci 9(9): 224. https://doi.org/10.3390/brainsci9090224

Morfoisse F, Renaud E, Hantelys F, Prats AC, Garmy-Susini B (2014) Role of hypoxia and vascular endothelial growth factors in lymphangiogenesis. Mol Cell Oncol 1(1): e29907. https://doi.org/10.4161/mco.29907

Opichka MA, Rappelt MW, Gutterman DD, Grobe JL, McIntosh JJ (2021) Vascular dysfunction in preeclampsia. Cells 10(11): 3055. https://doi.org/10.3390/cells10113055

Pandey AK, Singhi EK, Arroyo JP, Ikizler TA, Gould ER, Brown J, Beckman JA, Harrison DG, Moslehi J (2018) Mechanisms of VEGF (vascular endothelial growth factor) inhibitor-associated hypertension and vascular disease. Hypertension 71(2): e1-e8. https://doi.org/10.1161/HYPERTENSIONAHA.117.10271

Phipps EA, Benzing TR, Thandani TR, Karumanchi SA (2019) Pre-eclampsia: pathogenesis, novel diagnostics and therapies. Nat Rev Nephrol 15(5): 275–289. https://doi.org/10.1038/s41581-019-0119-6

Reshef T (2012) The role of hypoxia and hypoxia-inducible factor-1alpha in preeclampsia pathogenesis. Biol Reprod 87(6): 134. https://doi.org/10.1095/biolreprod.112.102723

Robinson ES, Khankin EV, Karumanchi SA, Humphreys BD (2010) Hypertension induced by vascular endothelial growth factor signaling pathway inhibition: mechanisms and potential use as a biomarker. Semin Nephrol 30(6): 591-601. https://doi.org/10.1016/j.semnephrol.2010.09.007

Ross C, Boroviak TE (2020) Origin and function of the yolk sac in primate embryogenesis. Nature Comm 11: 3760. https://doi.org/10.1038/s41467-020-17575-w

Salles AMR, Galvao TF, Silva MT, Motta LCD, Pereira MG (2012) Antioxidants for preventing preeclampsia: A systematic review. ScientificWorldJournal 2012: 243476. https://doi.org/10.1100/2012/243476

Sarkar AA, Sabatino JA, Sugrue KF, Zohn IE (2016) Abnormal labyrinthine zone in the Hectd1-null placenta. Placenta 38: 16-23. https://doi.org/10.1016/j.placenta.2015.12.002

Siragher E, Sferruzzi-Perri AN (2021) Placental hypoxia: What have we learnt from small animal models. Placenta 113: 29-47. https://doi.org/10.1016/j.placenta.2021.03.01

Situmorang PC, Ilyas S (2018) Description of testis histology of *Mus musculus* after giving nano herbal *Rhodomyrtus tomentosa* (haramonting). Asian J Pharm Clin Res 11: 461-463. https://doi.org/10.22159/ajpcr.2018.v11i11.29042

Situmorang PC, Ilyas S, Hutahaean S, Rosidah R (2020) Components and acute toxicity of nanoherbal haramonting (*Rhodomyrtus tomentosa*). J Herbmed Pharmacol 10: 139-148. https://doi.org/10.34172/jhp.2021.15

Situmorang PC, Ilyas S, Hutahaean S, Rosidah R (2021) Histological changes in placental rat apoptosis via FasL and cytochrome c by the nano-herbal *Zanthoxylum acanthopodium*. Saudi J Bio Sci 28(5): 3060–3068. https://doi.org/10.1016/j.sjbs.2021.02.047

Situmorang PC, Syahputra RA, Simanullang RH (2022) *EGFL7* and *HIF-1a* expression on human trophoblast placental by *Rhodomyrtus tomentosa* and *Zanthoxylum acanthopodium*. Pak J Biol Sci 25(2): 123-130. https://doi.org/10.3923/pjbs.2022.123.130

Strowitzki C, Taylor (2019) Protein hydroxylation by hypoxia-inducible factor (HIF) hydroxylases: Unique or ubiquitous? Cells 8(5): 384. https://doi.org/10.3390/cells8050384

Villanueva-Toledo JR, Chale-Dzul J, Castillo-Bautista C, Olivera-Castillo LA, Rangel-Méndez LA, Graniel-Sabido MJ, Moo-Puc RE (2020) Hepatoprotective effect of an ethanol extract of Tradescantia pallida against CCl4-induced liver damage in rats. S Afr J Bot 13: 444–450. https://doi.org/10.1016/j.sajb.2020.09.031

Vo T, Ngo D (2019) The health beneficial properties of *Rhodomyrtus tomentosa* as potential functional food. Biomolecules 9(2): 76. https://doi.org/10.3390/biom9020076

Wang HJ, Lu CK, Chen WC, Chen AC, Ueng YF (2019) Shenmai-Yin decreased the clearance of nifedipine in rats: The involvement of time-dependent inhibition of nifedipine oxidation. J Food Drug Anal 27(1): 284-294. https://doi.org/10.1016/j.jfda.2018.10.005

Zhang B, Kim MY, Elliot G, Zhou Y, Zhao G, Li D, Lowdon RF, Gormley M, Kapidzic M, Robinson JF, McMaster MT, Hong C, Mazor T, Hamilton E, Sears RL, Pehrsson EC, Marra MA, Jones SJM, Bilenky M, Hirst M, Wang T, Costello JF, Fisher SJ (2021) Human placental cytotrophoblast epigenome dynamics over gestation and alterations in placental disease. Dev Cell 56(9): 1238–1252.e5. https://doi.org/10.1016/j.devcel.2021.04.001

Zhang YB, Li W, Jiang L, Yang L, Chen NH, Wu ZN, Li YL, Wang GC (2018) Cytotoxic and anti-inflammatory active phloroglucinol derivatives from *Rhodomyrtus tomentosa*. Phytochemistry 153: 111–119. https://doi.org/10.1016/j.phytochem.2018.05.018.

AUTHOR CONTRIBUTION:

Contribution	Situmorang PC	Ilyas S	Siahaan DAS	Restuati M	Sari ER	Chairunisa C	Maliki MF
Concepts or ideas	x	x					
Design	x	x					
Definition of intellectual content		x	x	x			
Literature search			x		x	x	x
Experimental studies			x	x	x	x	x
Data acquisition	x	x					
Data analysis	x	x					
Statistical analysis	x						
Manuscript preparation	x		x	x			
Manuscript editing	x						
Manuscript review	x	x	x	x	x	x	x

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