Suppression of Fas expression in the hypertensive placental histology of rats given Nano herbal of *Rhodomyrtus tomentosa* leaves

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Abstract

High blood pressure during pregnancy can signal a high-risk pregnancy and result in difficulties. The expression of Fas is critical for maintaining hypertensive immunity. Fas is prevalent in the trophoblast and enhances maternal-derived apoptosis. Because it contains acylphloroglucinol, flavonoids, tannins, and triterpenes, *Rhodomyrtus tomentosa* (haramonting) is commonly used in traditional Indonesian medicine to treat high blood pressure. This study aimed to investigate and determine the role of haramonting in lowering Fas expression in hypertensive rats' placental histopathology. The rats were confirmed to be pregnant and divided into five groups: normal pregnant rats (control); hypertensive rats without treatment; and hypertensive rats given nanoherbal *Rhodomyrtus tomentosa (*NRT) at different doses: 100 mg/kg body weight (BW), 200 mg/kg BW, and 400 mg/kg BW. On gestation day 20, pregnant rats were euthanized under ketamine anaesthesia. Enzyme-linked immunosorbent assay (ELISA) analysis was used to examine the expression of the HSP family. Immunohistochemistry was used to assess Fas expression. In hypertensive rats, NRT lowered the systolic and diastolic blood pressure ($P < 0.01$), and NRT improved placental efficiency and restored birth weight. In hypertensive rats, higher doses of NRT were associated with higher levels of HSP27, HSP70, and HSP90. In placental histology, NRT suppresses Fas expression, acting as an anti-apoptotic agent in trophoblast cells. Because NRT is high in antioxidants and protects cells from hypoxia and dehydration, it suppresses Fas activity in the labyrinthine zone, basal zone, and yolk sac.

Keywords: Fas; herbal medicines; HSP; hypertensive; Immunohistochemistry.

1. Introduction

Hypertension, or high blood pressure, is considered a marker for the emergence of chronic inflammatory diseases and is an indicator of high-risk pregnancy. Hypertension in pregnancy can be mild, but if it is not handled properly, it can cause serious problems and threaten the lives of the mother and the foetus (McLaughlin *et al*., 2018). It is a generalized inflammatory disorder complicating gestation, with most of cases developing in the third trimester (Ali *et al*.,

2020). If chronic hypertension is not treated effectively during pregnancy, it can develop into chronic hypertension and preeclampsia. Protein in the urine is a symptom of hypertension, which can lead to complications, such as congestive heart failure, visual abnormalities, stroke, seizures, and kidney or liver disorders (McLaughlin *et al*., 2018).

Fas is a glycoprotein that causes apoptosis and is involved in the maintenance of tumour immunity as well as hypertension. Fas was shown to be a crucial molecule in the immune system's cell death signal transduction; it triggered apoptosis in Fas-positive cells through the binding of the Fas ligand to the extracellular domain of Fas (Ramezani *et al*., 2019). In the lipopolysaccharide (LPS) animal model, the activation of the Fas system and the induction of apoptosis can cause placental abnormalities (Ramezani *et al*., 2019). The extravillous trophoblast and the syncytiotrophoblast layer, the major sites of the feto-maternal interface, display consistent immunoreactivity to Fas in the placenta (Ejima *et al*., 2000). FasL's distribution pattern is nearly identical to that of Fas and Bcl-2 (Ejima *et al*., 2000). Fas is prevalent in the trophoblast, which contributes to immunity and causes apoptosis in maternally derived activated Fas-expressing cells. The Fas pathway can trigger apoptosis in placental cells during implantation (Ejima *et al*., 2000). Oxidants can regulate Fas-mediated cell death. In addition, both pharmacological antioxidants and antioxidant enzymes affect this process. Fas signalling induces ROS production, and Fas-induced activation of caspase-8 may be a target for redox regulation (Benhar, 2020). ROS generation was detected in cells sensitive to apoptosis but not in those inherently resistant to anti-Fas. Thus, the level of oxidative stress (arising from exogenous sources or endogenously generated upon receptor stimulation) was found to regulate the sensitivity of tumour cells or hypertensive cells undergoing Fas-mediated apoptosis.

This study used an antioxidant from *Rhodomyrtus tomentosa* leaves known in Indonesian as haramonting. This plant belongs to the *Myrtaceae* family and has woody stems (lignosus) with a habitus or shrub stature. The direction of stem growth is perpendicular (erectus), and the direction of branch growth is inclined upwards. The leaves are green and have a simple-leaf structure. This plant is often used in traditional Indonesian medicine to treat colic diarrhoea, dysentery, abscesses, bleeding, and high blood pressure and to improve smokers' lungs (Ilyas *et al*., 2019). Extracts from haramonting contain acylphloroglucinol, flavonoids, tannins, and triterpenes, and the plant exhibits high phenolic and antioxidant activity (Vo & Ngo, 2019). Haramonting leaves have antioxidant activity in vitro and in vivo (Jeong *et al*., 2013). Antioxidants from this plant can inhibit lipid peroxidation; improve the histology of the placenta, testes, and diabetic wounds; increase the expression of HSP70; and increase the ability to reduce free radicals (Ilyas & Situmorang, 2021; Ilyas *et al*., 2020; Situmorang *et al*., 2018a; Irianti *et al*., 2020). The high levels of phenolic compounds impart high antioxidant capacity; thus, haramonting is a potential source of health-promoting compounds (Zhang *et al*., 2018). This study aimed to determine and analyse haramonting's suppression of Fas expression in the histology of the placenta of hypertensive rats before investigating this process in human cells. Haramonting was converted to a nano-size to improve its penetration of and bioavailability in cells. It is hoped that this plant can be developed for molecular hypertension therapy in humans.

2. Materials and Methods

2.1 Materials

The leaves of haramonting (*Rhodomyrtus tomentosa*) were obtained in January 2021 from the yards of residents of Rantauprapat in the district of Labuhanbatu, North Sumatera Province, Indonesia. The plant was classified and authenticated by the Head of Botany at the Universitas Sumatera Utara and deposited into the Medanense Botanical Herbarium (registration range 6675/MEDAN/2021). Nanoherbal *R. tomentosa* (NRT) was produced at the Indonesian Research Institute (LIPI) in Jakarta, Indonesia, by high-energy milling (HEM) (Ilyas *et al.,* 2020; Situmorang *et al.,* 2021a). LPS was intraperitoneally administered within 3 hours using *Escherichia coli* 026:B6 (1 mg/kg BW) dissolved in 0.9% NaCl. Ketamine hydrochloride (30 mg/kg; Ketalar, Parke-Davis, Morris Plains, NJ, USA) was used as an anaesthetic before surgery. Santa Cruz Biotechnology provided anti-Fas (sc-715) antibodies (SanverTech, Heerhugowaard, the Netherlands). Cell Signaling Inc. provided mouse anti-phosphotyrosine and rabbit anti-Hsp27, anti-Hsp70, and anti-Hsp90 antibodies (Danvers, MA, USA).

2.2 Animal Handling

The experimental animals were pregnant female Wistar rats that weighed 150–200 g, were 2 months old, and were in a healthy condition (Situmorang *et al*., 2021a). This research passed the animal ethics review from the Ethics Commission of the Faculty of Mathematics and Natural Sciences, USU (ethical clearance no. 0259/KEPH-FMIPA/2021).

1. Acclimatisation: The rats were acclimatized for 2 months before being treated in the laboratory in an air-conditioned room with a 12-hour light/dark cycle. Male rats are separated from female rats (Ilyas *et al*., 2019)

2. Diet for the animals: Rats were fed conventional laboratory diets in the form of pellets and corn and given free access to water. Food was provided ad libitum (Ilyas *et al*., 2019).

3. Hypertension pregnancy: Each pair of Wistar rats was mated overnight and observed using the CCTV camera provided in the cage. Pregnancy was confirmed by a vaginal plug, and the day was defined as day 0. On gestational day 6, systemic inflammation was induced in pregnant rats by infusing *Escherichia coli* lipopolysaccharide (LPS) into the peritoneal cavity using a small osmotic pump (Irianti *et al*., 2020).

4. Surgery: The rats were sedated with ketamine on the 19th day of pregnancy. Then, surgery was performed to obtain the blood, foetus, and placenta (Situmorang *et al*., 2021a).

2.3 Design of experimental

This study used 50 pregnant rats (10 each in each group). The rats with confirmed pregnancy were divided into five treatments: (a) normal pregnant rats (C–), (b) hypertensive rats (C+), (c) hypertensive rats + NRT 100 mg/kg body weight (BW), (d) hypertensive rats + NRT 200 mg/kg BW, and (e) hypertensive rats + NRT 400 mg/kg BW. Pregnant rats were excised on gestational

day 20 under ketamine anaesthesia. The markers of hypertension were characterized by measuring blood pressure, and Heat Shock Protein (HSP) family expression was analysed by ELISA. Fas protein expression was evaluated by immunohistochemistry.

2.4 Measurement of systolic and diastolic blood pressure in pregnant rats

Blood pressure was determined before LPS injection and after LPS injection and NRT administration. Systolic and diastolic arterial blood pressure was recorded in conscious and uncontrolled animals for 1 hour, and the mean arterial blood pressure was measured using the non-invasive tail cuff method.

2.5 Enzyme-linked immunosorbent assay (ELISA)

The ELISA test from Enzo Life Sciences was used to perform quantitative HSP analysis. The HSP27 (rabbit) ELISA kit, HSP70 high-sensitivity ELISA kit, and HSP90 (rabbit) ELISA kit were utilised in this study. Following the manufacturer's recommendations, each sample was evaluated three times.

2.6 Hematoxylin-eosin (H&E) staining

The placenta in a formalin medium was removed from the storage area and placed in xylol for 15 min. Subsequently, the tissues were alternately hydrated in 96% and 70% pure alcohol, sequentially, for 5 minutes and then rinsed in distilled water. Haematoxylin dye was applied for 5 min, and then the tissues were rinsed for 3 min in distilled water. Eosin dye was added for 1 minute. The slides were dehydrated with 70%, 96%, and 100% alcohol, immersed in xylol, and placed on a cover glass. The sample was examined using a light microscope with 5 times the field of view (Irianti *et al.,* 2020).

2.6 Immunohistochemistry

To suppress endogenous peroxidase activity, 5 m thick paraffin-embedded placental slices were deparaffinized and treated with 1% H_2O_2 in methanol for 30 min before immunohistochemistry. Subsequently, the slides were rinsed in 0.01 M Tris-buffered saline (TBS pH 7.4). Tissue slices were treated with primary anti-Fas antibody (1:100 dilution in PBS containing 1% BSA) for 2 h after blocking non-specific binding to 1%skimmed milk in PBS. The Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) was used to identify immunoreactivity, which was counterstained with Mayer's haematoxylin.

2.7 Statistical analysis

Research data: Blood pressure and HSP family levels were analysed using a one-way analysis of variance with independent continuous variables and Duncan's multiple range test was used for comparison tests (Maramis *et al.,* 2015). Immunohistochemistry and tunnel assay was performed using Kruskal–Wallis and Mann Whitney Test on Sigmaplot software (Weiner & Craighead, 2010)

3. Results and discussion

3.1 Impact of nanoherbal *R. tomentosa* (NRT) on systolic and diastolic blood pressure

Table 1. The effects of hypertension on systolic and diastolic blood pressures with and without maternal NRT treatment

Groups	Systolic blood pressures (mmHg) (Mean ± SD)			Diastolic blood pressures (mmHg) (Mean ± SD)		
	Day 0	Day 6	Day 19	Day 0	Day 6	Day 19
C-	121 ± 8.02[a]	122 ± 9.12[a]	120 ± 5.99[a]	85 ± 2.34	90 ± 4.90[a]	87 ± 3.99[a]
C+	122 ± 7.71[a]	146 ± 9.23[b]	150 ± 6.76[b]	90 ± 4.01	118 ± 3.98[b]	120 ± 6.98[b]
NRT100	122 ± 4.54[a]	146 ± 3.56[b]	136 ± 7.55[ab]	89 ± 4.91	115 ± 5.88[b]	115 ± 5.87[b]
NRT200	123 ± 5.98[a]	150 ± 4.98[b]	130 ± 6.89[ab]	87 ± 4.89	116 ± 5.99[b]	100 ± 5.89[a]
NRT400	121 ± 7.78[a]	145 ±.7.22[b]	127 ± 6.22[a]	88 ± 8,21	115 ± 7.04[b]	95 ± 4.76[a]

Note: C-: Untreated, C+: Hypertension rat, NRT100: Hypertension rat given a dosage of 100mg/kgBW, NRT200: Hypertension rat given a dosage of 200mg/kgBW, NRT400: Hypertension rat given a dosage of 400mg/kgBW. NRT: Nano herbal *R.tomentosa,* Day 0: Before LPS Injection, Day 6: After LPS Injection, Day 19: After administration of NRT. Data were expressed as the mean ± standard deviation (The same letters denoting no difference).

NRT lowered blood pressure at all doses. No significant differences between treatments ($P > 0.05$) were observed on day 0; all animals had normal systolic and diastolic blood pressure. On the sixth day, all groups except the control group (C–) were given LPS injections, and a significant increase in systolic and diastolic blood pressure ($P < 0.01$) was observed compared with the control group (C–). NRT was administered to hypertensive rats for one week, from the 13th to the 19th gestational days, and lowered systolic and diastolic blood pressure ($P < 0.01$). Multiple comparisons indicated that NRT reduced hypertension, most likely because of NRT antioxidants such as glutathione, which is a potent antioxidant and can improve placental–uterine function (Wu, 2021). Glutathione is a natural antioxidant that aids in the preservation of all other antioxidants (Wu, 2021). Glutathione levels in the decidua were significantly higher than those in the placenta. Glutathione levels were higher in the decidua in patients with pre-eclampsia and patients with haemolysis, elevated liver enzymes, and low platelets (HELLP) than in patients with normotensive pregnancy, and only in the placenta of pre-eclampsia patients (Robaczewska *et al.,* 2016). Glutathione plays a role in combating oxidative stress and maintaining nitric oxide bioavailability via the formation of nitrosothiols and nitrosohemoglobin; disruptions in glutathione metabolism can cause an increase in blood pressure (Robaczewska *et al.,* 2016).

Under normal circumstances, antioxidants work to counteract ROS production by scavenging existing free radicals and promoting the repair of ROS-induced cell structure damage (Kudhur & Moustafa, 2018). At low levels, oxidative stress promotes some physiological reproductive functions; however, at high levels, it is linked to pathological processes in the reproductive tract that contribute to infertility and poor pregnancy outcomes

(Kudhur & Moustafa, 2018). Malondialdehyde levels in the plasma and placenta increased significantly, whereas glutathione and superoxide dismutase levels decreased significantly as diastolic blood pressure increased (Madazli *et al*., 2002). This Plant like *Morinda citrifolia* plays a pivotal role in controlling blood pressure (Purwaningroom *et al*., 2021). This research supports the findings of Irianti *et al*. (2020), which indicated that haramonting can lower the blood pressure in pregnant rats with preeclampsia while increasing the trophoblast count and improving placental histology.

3.2 Effects of Nano herbal *R.tomentosa* (NRT) treatment on birth weight

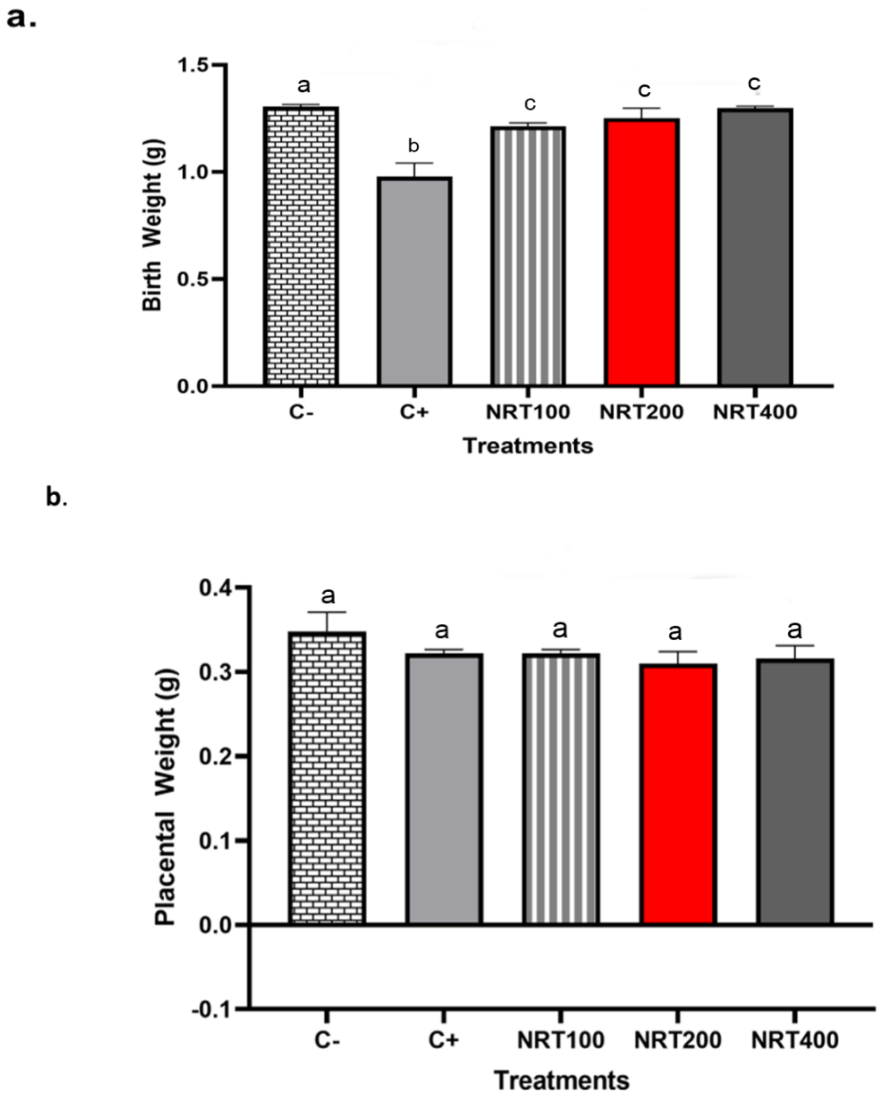

Fig. 1. Effects of Nano herbal *R.tomentosa* (NRT) treatment on fetal and placental weight, a.Birth Weight, b. Placental weight.
C-: Untreated, C+: Hypertension rat, NRT100: Hypertension rat given a dosage of 100mg/kgBW, NRT200: Hypertension rat given a dosage of 200mg/kgBW, NRT400: Hypertension rat given a dosage of 400mg/kgBW. Data were expressed as the mean ± standard deviation (The same letters denoting no difference)**.**

NTR treatment resulted in a significant difference in the birth weight of hypertensive rats ($P<0.01$). The multiple comparisons analysis revealed no significant differences at various NRT doses, but significant differences were observed when all NRT doses were compared with the C– and C+ groups. Hypertension can lead to foetal weight loss and disrupt the passage of nutrients from the mother to the foetus. However, the administration of NRT at the lowest to the highest doses demonstrated that this herb can restore foetal weight in hypertension ($P < 0.5$) (Figure 1a). It did not affect placental weight in healthy, hypertensive, or NRT-treated rats (Figure 1b). Pregnancy complications, including placental insufficiency, hypertension, and preeclampsia, slow the transport of nutrients to the foetus because of reduced placental and umbilical blood flow (Thornburg *et al.,* 2010). Therefore, intrauterine growth retardation, caused by reduced nutrition delivery to the foetus during pregnancy, is a significant health burden because of its associated perinatal morbidity and mortality (Thornburg *et al.,* 2010). One of the causes of the slowed transportation of nutrients and oxygen is the delayed growth of the foetus due to the development of cardiovascular and metabolic illnesses or hypertension (Thornburg *et al.,* 2010). Antioxidants can aid in the promotion of placental function while also lowering intrauterine growth. NRT can overcome this because it contains extremely potent antioxidants (Situmorang *et al*., 2021b). Perhaps NRT, like glutathione, plays a role in maintaining the biological value of germ cells; it has been linked to the fertilization process and early embryo development. Fortunately, glutathione can be recycled by the body if it is properly optimized, and it can also be destroyed (Aljaser *et al.,* 2021). Oxidative stress in the placenta and foetus may be exacerbated by a lack of nutrients and oxygen (Wu *et al*., 2012). The findings of this study confirm this theory, indicating that treating gestational hypertension with haramonting can increase placental efficiency and restore birth weight.

3.3 Effects of Nano herbal *R. tomentosa* (NRT) in Expression of HSP27, HSP70 and HSP90

HSP27 levels in hypertensive rats that received NTR therapy differed significantly from those of hypertensive (C+) rats ($P < 0.05$). The multiple comparisons analysis revealed that the 400 mg/kg NRT dose was associated with the highest HSP27 value, but the HSP27 levels associated with the 200 mg/kg doses was also significantly higher than those in the 100 mg/kg doses and C+ group. In hypertensive rats, higher doses of NRT were associated with higher levels of HSP27. HSP27 maintains intracellular redox homeostasis by maintaining glutathione in its reduced form and decreasing intracellular iron levels. As a result, it has anti-oxidative activity (Arrigo et *al*., 2005). NRT treatment induced high levels of HSP27 expression because it acts as a molecular chaperone, regulating the folding and renaturation of damaged proteins, which can occur after LPS and oxygen free radicals are injected. HSP27 also plays a role in physiological reactions to body fluid homeostasis, which influences blood pressure-regulating systems (Gostimirovic *et al*., 2020).

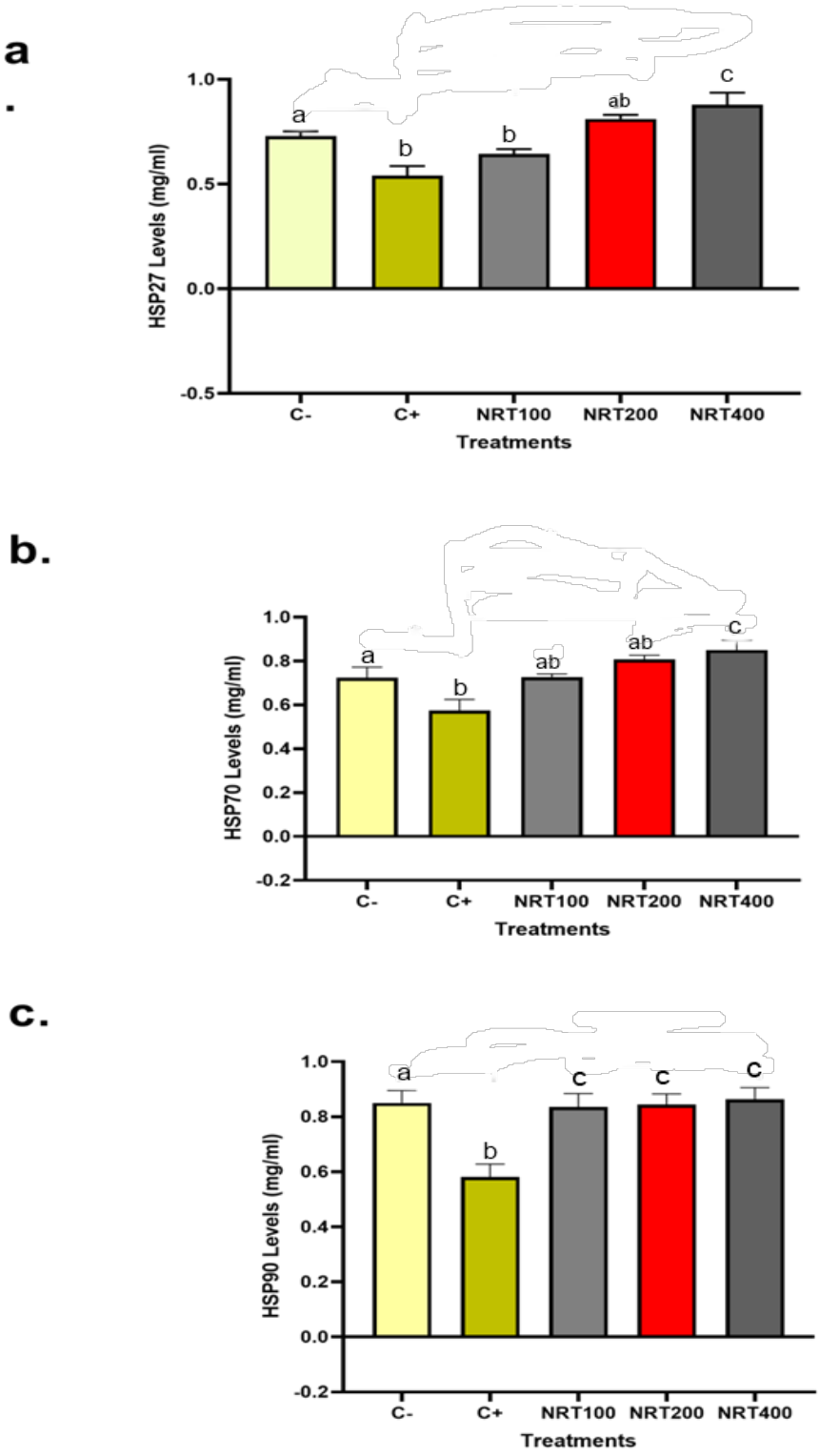

Fig 2. Effects of Nano herbal *R.tomentosa* (NRT) in Head Shock protein family, a. HSP27, b. HSP70, c.HSP90. C-: Untreated, C+: Hypertension rat, NRT100: Hypertension rat given a dosage of 100mg/kgBW, NRT200: Hypertension rat given a dosage of 200mg/kgBW, NRT400: Hypertension rat given a dosage of 400mg/kgBW. NRT: Nano herbal *Rhodomyrtus tomentosa*. Data were expressed as the mean ± standard deviation (The same letters denoting no difference).

HSP70 levels among hypertensive rats treated with NTR at dosages of 100 to 400 mg/kg BW differed significantly ($P < 0.05$). The multiple comparisons analysis revealed that the 100 NRT dose was associated with the highest HSP70 value, which was almost the same value as the 400 NRT dose group. Glutathione, which is similar to an antioxidant in NRT, regulates many cellular functions; it reduces hydrogen peroxide and peroxynitrite directly and indirectly with the help of glutathione peroxidase, detoxifies electrophiles, modulates reversible oxidation and reduction of protein thiols, and is crucially involved in the regulation of enzymes, transcription factors, and signal transduction (Oyagbemi *et al.,* 2018). HSP70 expression improves postischemic ventricular recovery, and overexpression of HSP70 in transgenic rats improves postischemic cardiac function and reduces neuronal cell death (Gostimirovic *et al*., 2020). LPS-induced protection in hypertension and inflammation is due in part to suppression of NF-kB activation and lymphocyte activation. Lipid peroxidation as well as superoxide and hydroxyl radical anion activity can be prevented and countered by the antioxidant action of haramonting (Situmorang *et al*., 2021b). Flavonoids in this plant extract can boost SOD and GSH-Px activity while also lowering MDA levels (Wu *et al*., 2015).

The multiple comparisons analysis revealed no significant differences ($P > 0.05$) between rats treated with NRT doses of 100 to 400 mg/kg BW. However, a significant difference was observed when all doses were compared with the C+ group ($P < 0.05$), as shown in Figure 2c. HSP90 is a key player in the signalling pathway that leads to eNOS activation. HSP90 regulates vasomotor activity in resistance vessels by inhibiting HSP90 signalling, which plays a role in modulating vasoactive responses in blood vessels (Jones *et al*., 2011). Increased HSP90 signalling promotes NO-dependent vascular hyporeactivity, indicating a link between protein–protein interactions in hypertension (Ai *et al*., 2003).

3.4 Fas expressions after given Nano herbal *R. tomentosa* (NRT) to placental rats

According to the findings of the histopathological examination (Figure 3), an increase in the number of syncytial nodes in all placental tissue preparations may be present in the mature placenta (Figure 3a), but syncytial nodes are also present in the hypertensive placenta (Figure 3b) because of the hypoxic state of the foetal placenta. Hypoxia causes the villi's ends or terminals to become irregularly shaped, increasing the likelihood of trophoblast accumulation and the creation of a syncytial knot. A shortage of oxygen perfusion caused an increase in the cytotrophoblast. In the placentas in the control and 400 mg/kg NRT groups, mature cytotrophoblast cells with a normal shape were formed, but the histology of rats with hypertension revealed that many new villi formed to support the lack of placental perfusion. The more trophoblast cells and new villi are formed, the more trophoblast cells and new villi are formed. Inadequate oxygen perfusion causes trophoblasts to expand and become cytotrophoblasts. The cytotrophoblast functions as the site of gas exchange, replacing the endothelial function of the arterioles (Staff *et al*., 2020). The absence or partial invasion of the spiral arteries by trophoblast cells causes vascular anomalies in hypertensive placentas. This invasion involves the replacement of musculo-elastic spiral artery walls with fibrinoid-filled walls. However, the administration of NRT at all doses (Figure 3c–e) began to improve these vascular abnormalities.

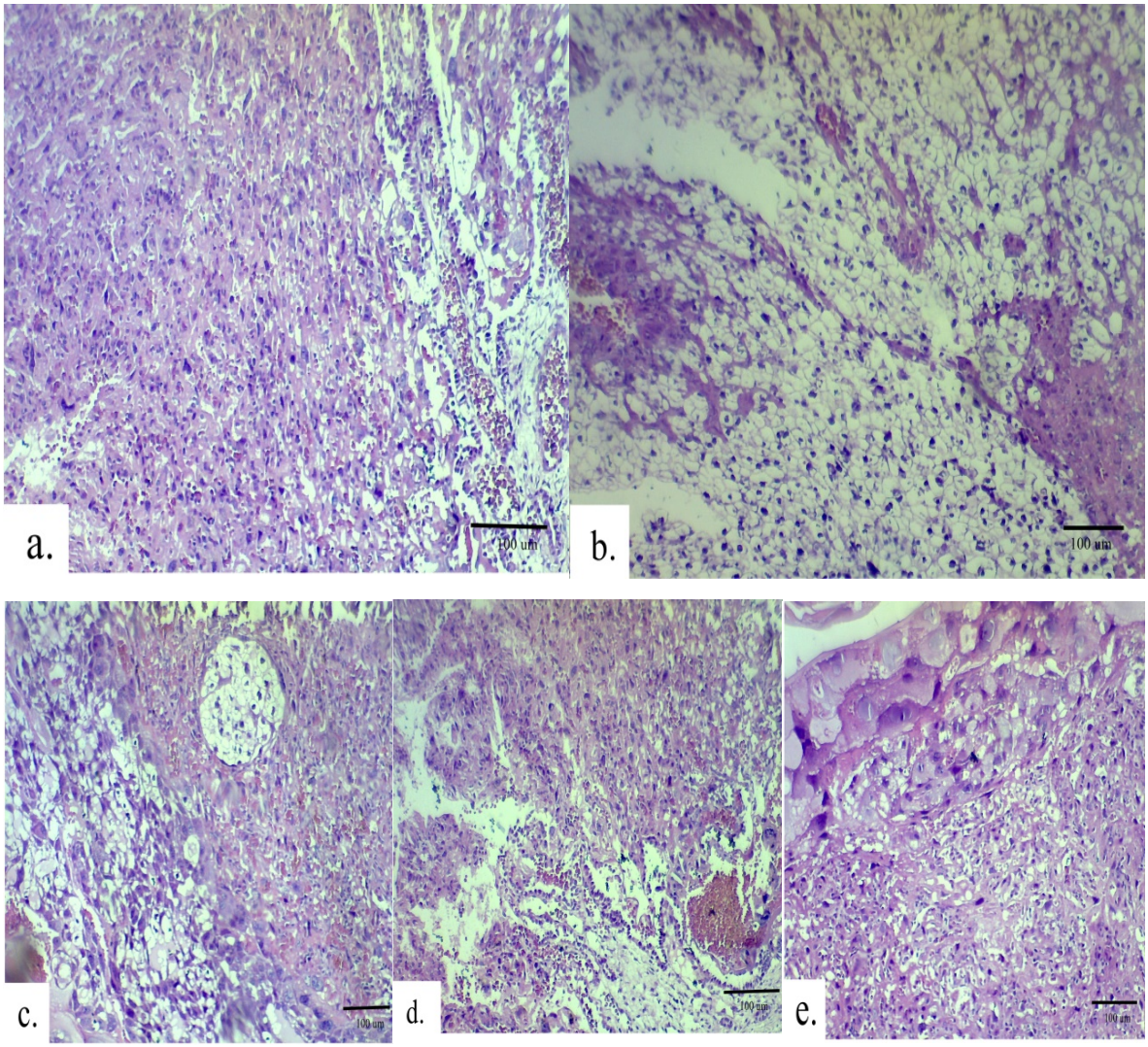

Fig. 3. Histology of placental hypertension by Nano herbal *R.tomentosa* (NRT)
a. Untreated, b. Hypertension rat, c. Hypertension rat given by NRT 100mg/kgBW, d. Hypertension rat given by NRT 200mg/kgBW, e: Hypertension rat given by NRT 400mg/kgBW (10x).

NTR treatment resulted in a significant difference in Fas expression in hypertensive rats ($P < 0.05$). The highest NRT dose resulted in a greater difference ($P < 0.01$). Lower Fas activity in hypertensive rats was associated with higher NRT doses (Table 2). In trophoblast cells, NRT suppression of Fas expression in placental histopathology may be anti-apoptotic (Figure 4). In humans, trophoblast tissue is involved in the regulation of maternal–foetal gas, nutrition, and waste product exchange. In rats, maternal–foetal exchange takes place in the labyrinthine zone, whereas placental hormone production takes place in the basal zone. An increase in Fas expression was observed in the basal zone in the hypertensive state, but Fas expression decreased with increasing doses of NRT (Figure 4). Caspase-8 may not be involved

in the rise in Fas in the histology of hypertension. The intrinsic apoptotic pathway can mediate increased caspase-3 and PARP levels (Staff *et al*., 2020). However, further research into the mechanism causing apoptosis-induced cell death in the basal zone is needed. The labyrinthine zone of hypertension histology, in contrast to the basal zone, reveals elevated expression of all Fas pathway-related proteins that activate death via caspase-8 (Staff *et al*., 2020). The yolk sac had the highest Fas expression revealed by placental histopathology in our investigation. The yolk sac is a nutrient transfer system that takes nutrients from the uterine gland secretions or the mother's blood. Most eutherian yolk sacs lose contact with the peripheral chorion ontogenetically, forming free splanchnopleuric yolk sacs that transport chemicals from the exocoelomic cavity (Zhao *et al*., 2018). Embryonic deformities, miscarriages, and growth problems can all be caused by errors in the yolk sac's development and function. In the labyrinth zone, basal zone, and yolk sac, NRT could decrease Fas activity (Table 2 and Figure 4). This is due to the presence of antioxidants such as anthocyanins, acylphloroglucinol, flavonoids, tannins, and triterpenes in NRT, which protect cells from hypoxia and death (Situmorang *et al*., 2021b; Situmorang *et al*., 2018b).

Table 2. Fas expression in histology of hypertension placental

Treatment	Labyrinth zone (Mean ± SD)	Labyrinth zone (Mean ± SD)	Yolk salc (Mean ± SD)
C-	12 ± 4.21^{a}	13± 5.89^{a}	20 ± 2.44 ^{a}
C+	32 ± 5.44^{b}	30 ± 4.21^{b}	61 ± 4.43^{b}
NRT100	22± 9.61^{c}	21± 5.22^{c}	31± 4.91^{c}
NRT200	20± 9.63^{bc}	19± 8.04^{ac}	22 ± 5.70^{d}
NRT400	14± 3.28 ^{a}	13± 7.32^{a}	15± 3.53^{a}

Note: C-: Untreated, C+: Hypertension rat, NRT100: Hypertension rat given a dosage of 100mg/kgBW, NRT200: Hypertension rat given a dosage of 200mg/kgBW, NRT400: Hypertension rat given a dosage of 400mg/kgBW. NRT: Nano herbal *R. tomentosa*. Data were expressed as the mean ± standard deviation (The same letters denoting no difference).

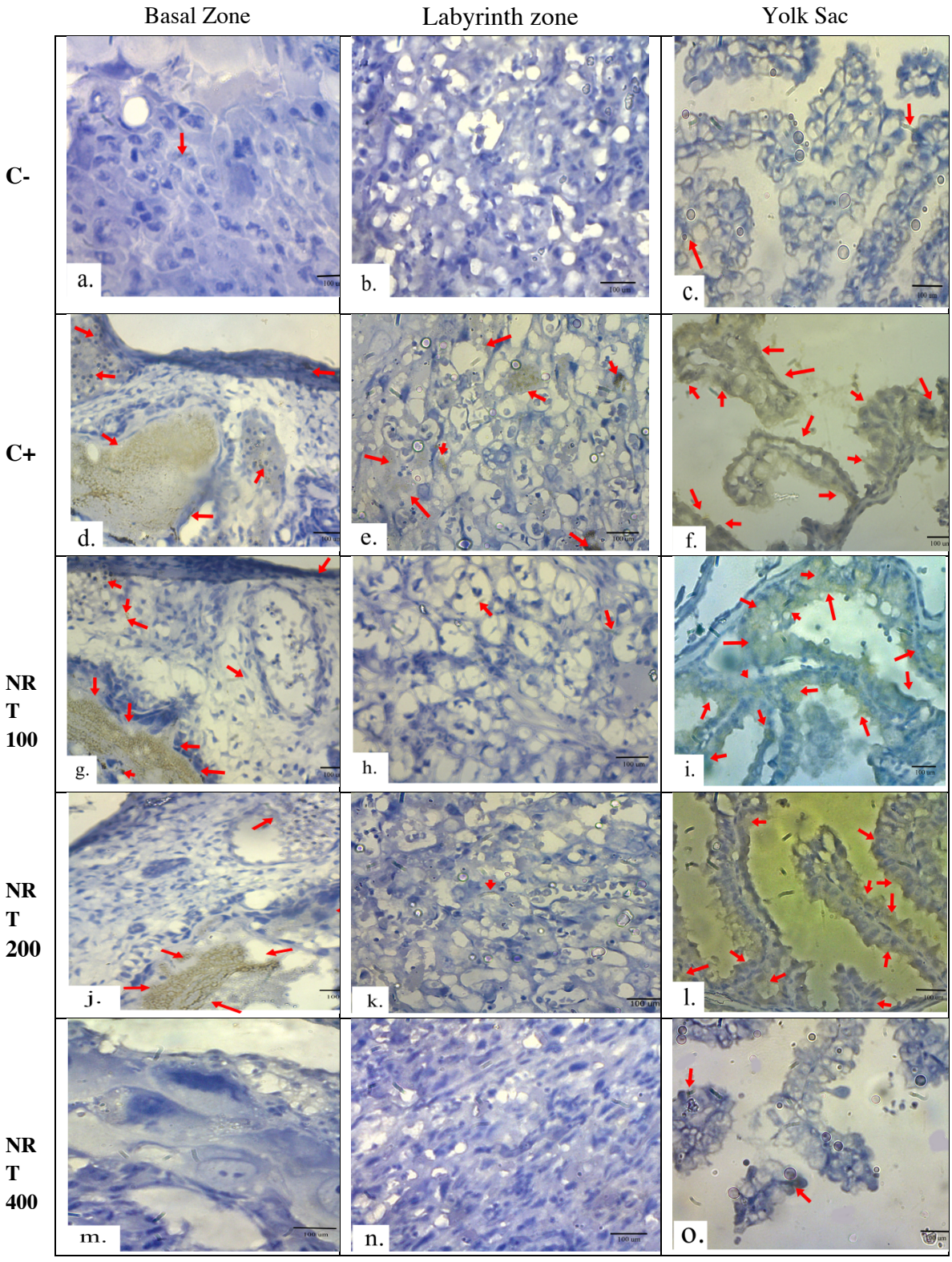

Fig. 4. Fas expression in hypertensive rat placenta histology. C-: Untreated, C+: Hypertension rat, NRT100: Hypertension rat given a dosage of 100mg/kgBW, NRT200: Hypertension rat given a dosage of 200mg/kgBW, NRT400: Hypertension rat given a dosage of 400mg/kgBW. NRT: Nano herbal *R.tomentosa* (40x).

4. Conclusion

According to the histopathological findings in this study, an increase in the number of syncytial nodes may be present in the mature placenta, but syncytial nodes are also present in the hypertensive placenta because of the placenta's hypoxic state. In contrast to the basal zone, the labyrinthine zone and yolk sac exhibit elevated expression of all Fas pathway-related proteins that activate death via caspase; the yolk sac had the highest Fas expression in our study. Embryonic deformities, miscarriages, and growth issues can all be caused by errors in the development and function of the yolk sac. Because of the presence of antioxidants, such as anthocyanins, acylphloroglucinol, flavonoids, tannins, and triterpenes, in nanoherbal *R. tomentosa* (NRT), cells in the labyrinth zone, basal zone, and yolk sack are protected from hypoxia and death. These findings demonstrated that hypertension caused a loss in placental efficiency in rats; a drop in HSP levels caused by hypoxia; and an increase in Fas expression, which thereby promoted the apoptotic pathway and resulted in a decrease in foetal birth weight. However, NRT treatment improved the placental function and birth weight in hypertensive pregnancy.

Conflict of interest

The authors declare that they have no conflict of interest

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