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Potential of pre−gestational intake of *Laportea interrupta* L. (stinging nettle) leaf decoction as an aid for fetal−maternal health

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ABSTRACT

Objective: To examine the potential of pre-gestational intake of *Laportea interrupta* L. (*L. interrupta*) leaf decoction as an aid for fetal-maternal health by determining its influence on embryonic implantation and growth, placental labyrinth vasculo-angiogenesis, and junctional zone morphology. **Methods:** Eight-week-old female mice were divided into groups and fed daily with 3.5 g food/mouse. The control was given drinking water, the treatment groups, low (LC), medium (MC), and high (HC) concentrations, were given 25%, 50%, and 100% v/v stock solution, respectively for 14 days, prior to mating. Pregnant mice were sacrificed at 14.5 days post-coitus. The uteri and placentae were collected and weighed; implantation sites were counted as either viable or resorbing. The estimated weight (g)/ embryo, as a function of the number of implantation sites, was evaluated. The histology of placental labyrinth angiogenesis and junctional zone morphology was examined. **Results:** The viable site ratios increased as leaf decoction concentration increased. This was most significant in the HC group ($P<0.05$). The HC group exhibited increase in the estimated embryo weight and implantation sites, and placental labyrinth with very prominent blood vessels. There was lesser depletion of junctional zone in all treatment groups with large blood vessels and glycogen cells that were more apparent in MC and HC group than those of the control and LC groups. **Conclusion:** Pre-gestational consumption of *L. interrupta* leaf decoction in high concentrations demonstrated its potential to support fetal-maternal health.

1. Introduction

Maternal health condition and placental development are main factors in the establishment of pregnancy, embryogenesis, fetal growth and survival at perinatal period[1]. The dense networks of blood vessels within the placenta are responsible for the exchange of respiratory gases, nutrients, and wastes between the mother and the embryo throughout pregnancy[2]. Placental vasculature constantly changes throughout pregnancy to facilitate rapid growth, and to supply the increasing metabolic needs of the growing embryo[3].

The rapid cell division during pregnancy also yields free radicals and other oxidative molecules which may exceed the available antioxidant buffering capacity of both the mother and the growing fetus, leading to cellular damage, abnormal placental growth, abortion, and some chromosomal defects[4,5]. This oxidative stress can also disrupt the vasodilation signaling process which is vital in the regulation of placental blood flow[6].

Adequate maternal nutritional condition before pregnancy, during pregnancy, and after delivery also plays a vital role in reducing adverse outcomes for both mother and infant[7]. Deficiency in micronutrients leads to maternal complications such as preterm labor (PTL), iron deficiency anemia, preeclampsia, intrauterine-growth restriction (IUGR), and small-for- gestational (SGA) infants[8].

As of 2013 report of Philippine Statistics Authority[9], total fetal deaths in the Philippines increased by 1% from 2010 to 2011. The main cause of fetal death (55.1%), comprising 4 503 cases was reported to be due to disorders related to short gestation and

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low birth weight (LBW). The other leading causes are intrauterine hypoxia and congenital malformations. These top three leading causes of fetal deaths were consistent from 2009-2011. Maternal deaths also increased from 162 per 100,000 childbirths in 2006 to 221 in 2010. With this trend, it is still unlikely to meet the Millennium Development Goal (MDG) of the Philippines to reduce maternal deaths by 2015[10].

In a developing country like the Philippines, the high cost of medications, treatments, and supplements for pregnancy are inaccessible for the majority of the population. This calls for alternative means such as herbal remedies.

Nettle is one of the most commonly recommended herbal remedies to aid in pregnancy in traditional medicine. Nettle belongs to the family Urticaceae which is claimed to contain significant levels of minerals such as iron, manganese, calcium, potassium, and Vitamins A, C, D and E[11]. It is usually prepared as tea by boiling fresh or dried leaves and also consumed as vegetable.

The most popular nettle species that are used to make nettle tea are *Urtica dioica* (*U. dioica*) and *Urtica urens* (*U. urens*). However *Laportea interrupta* (*L. interrupta*) and *Boehmeria nivea* (*B. nivea*) are the species most commonly found in the Philippines. While *B. nivea* tea is already widely used in China to treat miscarriages, excessive menstrual flow, and abnormal placental movements[12], no studies have been conducted on the potential effects of *L. interrupta* on pregnancy.

L. interrupta, commonly known as nettle or Lipang-aso in Tagalog, is a particularly undervalued plant in the Philippines. Since it is considered a weed and contains stinging hairs, it is usually uprooted or avoided. However, it has been used traditionally in the Philippines as remedy for muscle pains, carbuncles, asthma and cough[13]. In other countries, *L. interrupta* is also used in conditions such as amenorrhea, female hormonal imbalance, menorrhagia, osteoporosis, premenstrual syndrome, prostate diseases[14], urinary diseases[15], impotency, and spermatorrhea[16].

In this study, the potential of *L. interrupta* in establishing pregnancy and supporting maternal and fetal health was evaluated. Results from this study can support the claim that this plant can be used as a low-cost alternative means of nutrient and antioxidant supplementation for pregnancy.

2. Materials and methods

2.1. Test animals and set–up

Twenty-four (24) six-week-old ICR female mice, weighing an average of about 25 g were purchased from University of the Philippines. Diliman, Marine Science Institute, Quezon City. Twelve (12) sexually mature virile mice, approximately 15-18 week-old were used as studs. These were kept in the animal house of De La Salle University – Manila at 26 ℃ temperature under 12 h light: 12 h dark cycle. The cages were sanitized on a weekly basis, the wood shavings autoclaved, plates washed and dried twice a week, and water bottles were washed and filled every other day. Acclimatization lasted for two weeks, at which time the female mice were already sexually mature. Food pellets and water were provided *ad libitum* during acclimatization. Proper handling and maintenance of these animals adhered to the guiding principles of handling laboratory animals in accordance with Veterinary Medical Association's safety standards of the Philippines.

2.2. Collection and preparation of L. interrupta leaf decoction

Plants were collected from a residential back yard in Niugan, Angat, Bulacan located exactly at 14.945459 latitude and 120.970894 longitude. The plant was authenticated taxonomically by a plant taxonomist of the Philippine National Museum. Collected leaves were washed with running water and freeze-dried at the instrument room of the Chemistry Department of De La Salle University- Manila. Freeze-dried leaves were ground using an electric grinder and kept in an air-tight container at -4 ℃. To prepare the stock solution, 1.0 g leaves was boiled in 100 mL purified drinking water in an Erlenmeyer flask for 30 min. The decoction was filtered with cheesecloth and transferred in a beaker. Three decoction concentrations were prepared from the stock solution (1 g leaves/100 mL purified drinking water) by dilution in cold purified drinking water: 25% v/v, 50% v/v, and 100% v/v designated as low, medium and high concentration, respectively. A decoction was prepared every other day to preserve potency as well as to minimize possible spoilage.

2.3. Treatment administration

Female mice were randomly divided into 4 groups - 1 control and 3 treated groups: low (LC), medium (MC) and high concentration (HC) groups. Control group was given purified drinking water as liquid source. LC, MC and HC groups received 25% v/v, 50% v/v, and 100% v/v decoction concentrations, respectively. Treatments were administered by initially supplying each mouse with 1.5 mL of water (for control) and decoction (for LC, MC, and HC) with 1 mL of 2% v/v honey solution (1ml honey:50ml water) to ensure complete consumption. As soon as consumed, mice were given drinking water ad libitum within the next hours. Treatment administration lasted for 14 days which commenced after the acclimatization period and culminated before the mating. This was done daily during the 14-day period.

2.4. Mating

After the 14-day supplementation period, 1 male mouse and 2 female mice were joined together in one cage at 1700 hours. Female mice were monitored the following day at 0700 hours for the presence of vaginal plug which determines the 0.5 day of pregnancy. Each pregnant mouse was kept in their respective cages.

2.5. Uterus extraction, histological slide preparation, and data collection

Female mice were sacrificed through cervical dislocation on 14.5 dpc. Uteri of the euthanized mice were dissected out and weighed using a digital scale. The number of viable implantation sites and resorption sites were counted after fixing the uteri in 10% formalin. The fixed samples were brought to the Histopathology Laboratory of Philippine Kidney Dialysis Foundation, Quezon City, Philippines for standard histological processing and slide preparation using hematoxylin and eosin stain (H&E). The viable implantation site ratio and estimated weight per viable implant for each female mouse were calculated using the formulas. Weights from uteri with the same number of viable implantation sites were obtained for each group.

After obtaining the prepared histological slides, angiogenesis in the placental labyrinth, and junctional zone morphology were qualitatively examined at 22 x magnification using Celestron Digital Microscope connected with ASUS K45V Laptop. Photomicrograph of each placenta was documented in the connected device.

2.6. Statistical analysis

Mean ratio of viable site was expressed as mean values ± SD. These were evaluated using One-way Analysis of Variance (ANOVA) followed by Tukey Honest Significant Difference (HSD) test using VassarStats. Values of P< 0.05 were considered significant. Microsoft Excel 2010 was used to evaluate the linear relationship trend between number of implantation sites and weight per viable implantation site, as well as determining the coefficient of determination (R2) for each group.

3. Results

3.1 Mean ratio of viable implantation sites

Among the 24 sacrificed female mice, 21 were found successfully impregnated. At an increasing concentration of *L. interrupta* decoction, there was an observed reduction of resorbing embryos. Consequently the mean ratio of viable implantation sites increased. In the HC group, all implantation sites were viable while the control group yielded the lowest mean ratio of viable implantation sites (Table 1). HC group was found statistically significant from the control group (P<0.05). However, it is not significantly different from all the other treatment groups.

Table 1

Comparison of total number of implantation sites and mean viable site ratios in pregnant mice from all groups.

Parameters	Control group n=6	LC group(25% SS) n=4	MC group(50% SS) n=4	HC group(100% SS) n=5
Total number of implantation sites	67	42	43	43
Mean viable site ratio (%) SD	85.40 ± 10.22[a]	92.18 ± 5.59[a]	94.50 ± 6.89)[a]	100.00 ± 0.00[b]

Values with different letters are significantly different from each other (P<0.05), n = number of uteri.

3.2 Estimated weight per viable embryo

The estimated weight per embryo as a function of number of viable implantation sites in the control, LC group, MC group, and HC group is shown in Figure 1 with their respective coefficient of determination (R2). All groups except HC group exhibited an inverse relationship between the two variables. That is, the higher the number of implantation sites, the lower the weight per embryo.

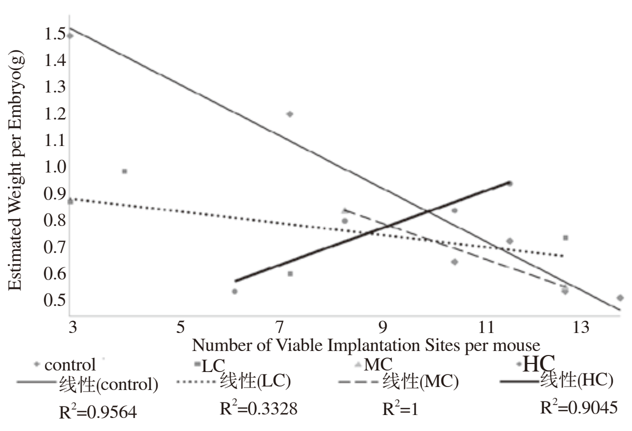

Figure 1. Estimated weight per embryo as a function of number of viable implantation sites per mouse.

Strong correlation between number of viable implantation sites and weight per embryo was observed in the control, MC, and HC groups. There was also an observed reduction in the steepness of slope between the control and MC group.

3.3. Placental labyrinth vasculo–angiogenesis

All groups exhibited normal vascular network formation in the placental labyrinth zone. However there was an apparent increase in vasculo-angiogenesis in the HC group which was evident by more prominent blood vessels as compared with the other groups (Figure 2, 3).

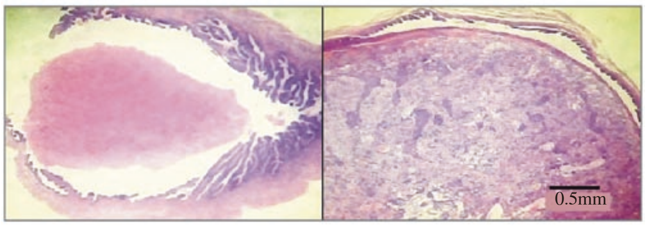

Figure 2. Comparison of placentae from a resorption site (left) and a viable site (right) at 22× magnification.

3.4. Junctional zone morphology

All treatment groups exhibited relatively wider junctional zones compared to the control group (Figure 4). Abundance of glycogen cells and larger maternal blood vessels were also observed in all the treatment groups. However these were more apparent among MC and HC groups.

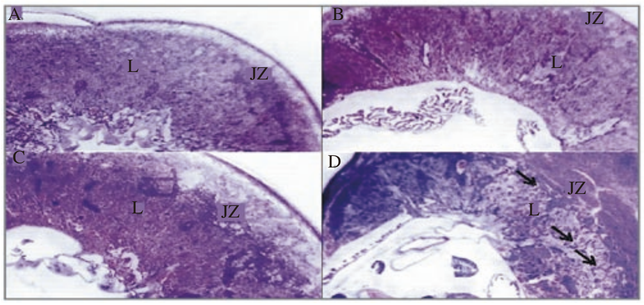

Figure 3. Cross-section of placenta from all groups showing the labyrinth at 22× magnification. (A) Control group (B) LC group (C) MC group (D) HC group (L=Labyrinth, JZ = Junctional zone, arrows=blood vessels).

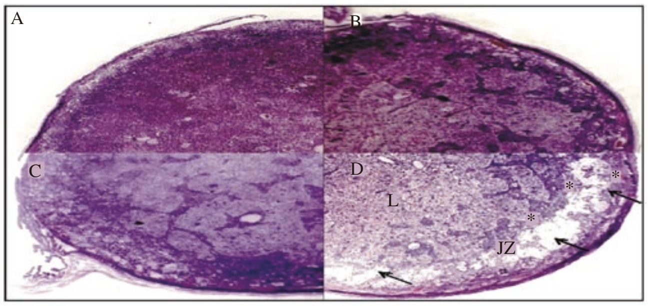

Figure 4. Comparison of placenta from all groups showing the junctional zones.
(A) Control group (B) LC group (C) MC group (D) HC group
(L = labyrinth, JZ = junctional zone, asterisk = glycogen cells, arrows = maternal blood vessels).

4. Discussion

The possible causes for the observed lower mean ratio of viable sites and higher resorption rate in the control group might include nutrient deficiency, elevated oxidative stress, and placental insufficiency. Undernutrition during pregnancy significantly reduces the number of infants in mice, by increasing the phenomenon of embryo resorption and neonatal mortality[17]. Deficiency in nutrients such as folic acid[18], arginine[19], copper[20] and Vitamin E[21] was also proven to cause embryo resorption. During pregnancy, the mother must provide a source of nutrition and gaseous exchange while also undergoing preparation for parturition and lactation. Insufficient supply of nutrition results to competition between the mother and the embryos which is detrimental for both[22].

Oxidative stress has also been suggested as a causative agent of resorption[23, 24]. Uncontrolled generation of reactive oxygen species (ROS) beyond physiological antioxidant defenses may lead to embryo resorption and placental degeneration[25] which can be linked with the observed incomplete placental development in the implantation sites of resorbing embryos (Figure 2).

Embryo resorption is expected and found in most strains of mice[26]. It is a common event particularly in multiparous animals due to the incapacity of the mother to sustain fetal growth and development. Increase in resorptions is indicative of decline in reproductive capacity of mice, rats, hamster and other multiparous species[27]. It also serves as a natural mechanism to eliminate embryos which are unfit for survival. In a study conducted by Kalter[28], reduced number of malformed embryos was associated with increasing resorption.

The observed absence of embryo resorption in HC group might be due to the elevated concentration of nutrients and antioxidants present in the nettle leaf decoction which could be adequate for all the implanted embryos. Results of studies conducted on *L. interrupta* support its nutriceutical potential. Krishna *et al*. [29] evaluated the nutritional, antioxidant, and antipyretic properties of *L. interrupta* wherein results show that its leaves contain significant amount of carbohydrates, proteins, starch, essential amino acids, and minerals. It was also revealed to contain high total phenolic and flavonoid contents. Its observed radical scavenging capacity and ferric reducing ability also show that it has antioxidant properties[14]. Thus *L. interrupta* may supply additional antioxidants that can cross the placenta and help combat oxidative stress. Oxidative stress induces the placenta to release anti-angiogenic and apoptotic factors[3] that may have led to failure in developing a functional placenta and to the resorptions observed in all groups except HC group. As pregnancy progresses, increased blood flow in the placenta is also required. Excessive free radicals in the blood can also cause disruption in the vasodilatation signalling process leading to decreased blood flow to the placenta and inefficient exchange of materials[30].

Average consumption of herbal infusions such as chamomile, mint, and nettle tea was already proven to be non-significant source of nutrition[31]. However, a 100% stock solution which was prepared using 1g of freeze-dried leaves and 100 ml water can be more concentrated than infusions. Consumption of a more concentrated decoction could result to a more significant effect.

Reduction of weight per embryo at increasing number of implantation sites is normal in multiparous animals due to the division of limited resources that the mother can provide. An additional embryo demands a proportionate increase in the nutritive level of the mother. This also means that the relative decrease in mean weight of each embryo will be greater in inverse proportion for each additional individual in the litter, as a result of a proportionate reduction in the nourishment available for each embryo[22]. Thus negative slopes observed in the trend lines of the control, LC, and MC groups using the number of viable implantation sites as a function of weight per embryo corresponded to the general assumption. In contrast HC group exhibited an increasing trend, which conforms to the absence of resorbing sites discussed previously.

It is generally presumed that the mean weight per embryo exhibits modifications as a function of age of mother, inbreeding, litter rank, and nutritional state[22]. In the HC group, growth was not restricted despite the increasing number of viable implantation sites. The mother was able to provide the nutritional and metabolic needs of the growing embryo despite the additional embryos. This also suggests efficient maternal-fetal exchange of materials. A study conducted by Richter *et al*. [32] also suggests that maternal antioxidant treatment can increase birth weight by improving placental function and securing fetal growth.

The observed reduction in the steepness of slope between the control and MC group suggests concentration-dependent outcome similar to the observed mean ratio of viable implantation sites. The increase in weight per embryo at increasing number of litter size observed in HC group exceeded the expected optimal outcome which is a consistent weight at any number of implantation sites. This may require an increase in sample size.

The same principle behind reduction of resorbing site relating antioxidant levels and angiogenesis can be applied in the apparent increase in vasculo-angiogenesis in the HC group. The placenta releases both anti-angiogenic and pro-angiogenic factors to regulate the formation of placental vasculature. Aside from favoring the release of anti-angiogenic factors, excess free radicals that cause oxidative stress attack the endothelia of blood vessels by acquiring electrons and denaturing DNA[4]. Thus supplementation of possible additional antioxidant from the nettle leaf decoction may have promoted placental vasculo-angiogenesis by inhibiting the favored release of anti-angiogenic factors. Morphological studies have shown that poor placental vasculature development is associated with fetal growth restriction[33]. Hence the formation of a more complex placental vasculature observed in the labyrinth of HC group may also be accounted for the direct relationship observed between weight of the embryos and number of implantation sites in the HC group.

Reduction in the relative volume of the junctional zone normally occurs as pregnancy progresses. This takes place as a compensatory mechanism to the increased volume of labyrinth zone volume as more vascular network is formed for fetal-maternal exchange. The glycogen cells in the junctional zone also diminish as more glycogen is consumed due to increased metabolism at the later stages of pregnancy. However the complete absence and extreme reduction of junctional zone is proven to cause fetal death[34, 35]. Thus, reduction of junctional zone depletion is beneficial for the growing embryo.

Abundance of blood vessels favors increase in surface area of fetal-maternal exchange of materials while larger blood vessels favor increased blood flow[36]. As more blood is supplied in the labyrinth zone from the maternal blood vessels in the junctional zone, increased angiogenesis will also be beneficial for optimal distribution and exchange of materials. This was consistent with the observed increase in angiogenesis in the HC group.

Increase in oxygen levels which may result from increase in placental blood flow may have also assisted in balancing antioxidant and ROS levels. Oxidative stress within the placenta caused by ROS is also important as it acts as a signaling pathway to influence the expression of transcription factors such as KLF8, Ets-1, NF B, Sp1, Sp3, STAT-3, and Nrf2 that regulate the expression and activity of proteins related to angiogenesis[30]. Oxygen readily reacts with other radicals and also generates free radicals which are also produced from normal metabolic processes in the body[37].

Pre-gestational consumption of *L. interrupta* leaf decoction in high concentrations is capable of positively influencing placental angiogenesis and morphology, fetal growth, and maternal health by preventing antioxidant and nutritional depletion and helping restore the balance to oxidative pathways.

Declare of interest statement

The authors declare that there is no conflict of interests.

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