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Asiatic acid in *Centella asiatica* extract towards morphological development in an intermittent hypoxia intrauterine embryo model and molecular prediction pathway of insulin-like growth factor-1 receptor signalling

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Abstract

Background: Hypoxia during pregnancy generates oxidative stress that alters the growth and development of the human fetus. Insulin-like growth factor-1 (IGF-1) receptors are essential for normal fetal growth. Asiatic acid in *Centella asiatica* (CA) has antioxidant properties to prevent growth impairment in hypoxia.

Aims: This study aimed to investigate the effect of asiatic acid on the morphological development of an intermittent hypoxia (IH) zebrafish embryo model and analyze molecular docking prediction in IGF-1 receptor (IGF-1R) signaling.

Methods: Embryos of zebrafish at 2 hours postfertilization (hpf) were assigned to control negative (C), IH, and combination IH and CA extract groups consisting of 1.25 (IHCA1), 2.5 (IHCA2), and 5 (IHCA3) µg/ml. Hypoxia treatment (conducted 4 hours/day) and CA extract were administered for 3 days (2–72 hpf). The parameters of body length and head length were evaluated at 3, 6, and 9 days postfertilization (dpf). The data were analyzed by a two-way analysis of variance ($p < 0.05$). Molecular docking was performed to explore the binding affinity of asiatic acid to IGF-1R by Molegro Virtual Docker ver.5 software.

Results: The body length and head length of embryos in the IH and treatment groups (IHCA) were shorter than those in the control group at 3 dpf ($p < 0.05$). However, the body length was more prolonged in the IHCA1 group, but the head length was longer in the IHCA2 group than in the IH group at 6 and 9 dpf. Molecular docking showed the reliable interaction of asiatic acid with IGF-1R signaling in an IH animal model.

Conclusion: The administration of CA extract benefits IH through the development and growth of zebrafish embryos at a dose of 2.5–5 µg/ml. Asiatic acid has a binding affinity for IGF-1R signaling.

Keywords: Asiatic acid, *Centella asiatica*, Zebrafish embryo, Morphology, IGF-1R.

Introduction

Oxidative stress may link altered placental function to fetal programming (Rodríguez-Rodríguez *et al.*, 2018). Fetal programming occurs when the usual pattern of fetal growth in humans or animals is disturbed by a deviant stimulus, such as inadequate nutrition and hypoxia (low ambient oxygen), which can occur at a crucial period during intrauterine life (Kwon and Kim, 2017). The hypoxic state of pregnancy is highly implicated in fetal programming by interfering with the development of the placenta and fetus (Fajersztajn and Veras, 2017). During fetal development, hypoxia is involved in several embryonic processes, including placentation, angiogenesis, and haematopoiesis (Perrone *et al.*, 2016). Hypoxia during pregnancy can develop from several maternal conditions, such as maternal smoking, anemia, placental insufficiency, hypertension, asthma, and respiratory tract infection (Jang *et al.*, 2015).

Hypoxia is also involved in the pathogenesis of other human diseases, such as tumorigenesis (Kamei and Duan, 2018). Hypoxia is one of the most significant stressors affecting aquatic ecosystems globally, and its severity and incidence are expected to rise due to increased nutrient intake and climate change (Fitzgerald *et al.*, 2016). In rats, prenatal hypoxia impairs endothelium-dependent relaxation in the mesenteric circulation of the newborn child (Fowden *et al.*, 2006). Malnutrition, stress, and hypoxia can alter the concentrations of hormones that cross the placental

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barrier in the mother and fetus, including growth hormone (GH), insulin-like growth factor (IGF), insulin, glucocorticoids, catecholamines, leptin, thyroid hormone, placental hormone eicosanoids, sex steroids, and placental lactogen (Fowden *et al.*, 2006). IGF-1 is synthesized in the liver via the pituitary/liver-GH/IGF-1 system axis, which also involves GHs. IGF-1 and its receptor (IGF-1R) are expressed in various extrahepatic organs (Acharjee *et al.*, 2017). The placenta secretes IGF-1 during pregnancy. IGF-1 then stimulates the transfer of essential nutrients from the placenta's mother to the fetus (Hellström *et al.*, 2016). IGF-1 plays an important role in normal fetal growth and stimulates fetal cell proliferation, differentiation, and synthesis of protein and glycogen mediated via IGF receptors and binding proteins (Malhotra *et al.*, 2019).

Hypoxia induces oxidative stress; thus, administering antioxidants provides beneficial outcomes (Merino *et al.*, 2014). The health benefits of Centella *asiatica* (CA) are fighting free radicals, reducing inflammation, protecting nerve cells, and accelerating wound healing (He *et al.*, 2023) by triggering blood microcirculation and collagen formation. Other benefits are diarrhea and stomach ulcers (Mishra *et al.*, 2022). CA was previously reported to have antioxidant properties and protect rotenone-induced zebrafish embryos from oxidative stress (Cory'ah *et al.*, 2020). A previous study demonstrated that CA extracts at concentrations of 1.25–5 µg/ml could enhance body length and locomotor activity in zebrafish larvae in the chronic constant hypoxia model (Ariani and Widiyastuti, 2022). The bioactive compounds from CA include triterpenoids, phenolic compounds, essential oils, pectins, free amino acids, vitamins, and minerals (Tripathy and Srivastav, 2023). The most dominant triterpenoids for health are asiatic acid, madecassic acid, asiatic acid-coside, and madecassoside (Gray *et al.*, 2018). Asiatic acid is known as a strong anti-inflammatory agent. tory and wound healing abilities as well as regulating the effect of apoptosis in several chronic diseases, such as neurodegeneration, cancer, and hypertension (Kukula *et al.*, 2022). Asiatic acid, an active compound in CA and the family of triterpenoids, is a potent cellular antioxidant (Wang *et al.*, 2021) that may have activity on IGF-1. Zebrafish embryos are proposed as a commonsense model for pediatric research according to their superiority compared to other animals. This study investigates the effect of asiatic acid in intermittent hypoxia (IH) zebrafish embryos and analyses the predicted interaction pathway of asiatic acid with IGF-1R signaling.

Material and Methods

Materials

Adult wild-type zebrafish were obtained from the Faculty of Fisheries and Marine Science, Universitas Brawijaya. The simplica of CA leaves was obtained from Materia Medica, Malang, East Java, Indonesia. All reagents and chemicals were analytical grade and obtained from E-Merck.

Experimental design

Adult zebrafish of both sexes were kept in a 60-l glass aquarium with a female and male ratio of 2:1. Adult fish were maintained at a density of 4–5 fish l^{-1} (Mikloska *et al.*, 2022). The temperature was set at 28°C ± 1°C, and the light/dark cycle was 14:10 hours. Food was provided twice a day with TetraMin (TetraMin Tropical Fish Flakes, USA) (Robertson *et al.*, 2014). Embryo collection was carried out following the beginning of the light period. Egg water was replaced twice a day. Neither eggs nor embryos were reared for 12 hours:12 hours in light: dark radiation (Mikloska *et al.*, 2022).

This research was a true experimental study with a posttest-only control group design approach. A total of 20 embryos at 2 hours postfertilization (hpf) were involved in each group of experiments. Every group contained three replications (Fitzgerald *et al.*, 2016). The experiments consisted of five groups: a control group (normoxia), IH, and IH + extract of CA (µg/ml) at 1.25 (IHCA1), 2.5 (IHCA2), and 5 (IHCA3) (Darwitri *et al.*, 2018).

Intermittent hypoxia

Hypoxia was performed by exposing the embryos to a water chamber that supplied nitrogen gas to reach oxygen pressure PO_2~5 kPa, dissolved oxygen (DO) 2 mg O_2/l as hypoxia status. Hypoxia was applied as an intermittent schedule at 2, 24, 48, and 72 hpf for 4 hours of exposure (at 08.00–12.00 am) followed by normoxia (PO_2~20 kPa, DO 8 mg O_2/l) for the rest through medium substitution. A DO meter tightly monitors the oxygen concentration (Kamei and Duan, 2018).

Preparation and treatment of CA extract

Simplicia of 100 g of CA was macerated in 900 ml of 96% ethanol for 24 hours, followed by filtration. This procedure was replicated three times, and then the filtrate was collected for further rotary evaporation. The crude extract was obtained from pasta, diluted in normal saline, and stored at 0°C. Preliminary research showed that based on the liquid chromatography mass spectrometry test, one of the components in the ethanol extract of CA is acetic acid. The extract was dissolved in an embryo medium that contained a mixture of embryonic medium and CA extract (Darwitri *et al.*, 2018). The medium-containing extract solution was administered simultaneously from 2 until 72 hpf, with IH in the treatment groups. The medium was changed every day.

Body length and head length measurement

After hatching, the larvae were kept in an embryonic medium and monitored for body and head length measurements at 3, 6, and 9 days postfertilization (dpf). Body length was measured from the tip of the head to the end of the trunk (before the caudal fin) (Collery *et al.*, 2014). The head length was scaled from the snout to the operculum. The body length and head length were measured using Image Raster software. The observation was conducted under a stereomicroscope (Olympus SZ61) connected to an Optilab camera and Optilab software version 2.0.

Molecular docking analysis

The protein sequence of the IGF receptor (GenBank ID NP_694501.1) was retrieved from the GenBank database and then modeled by SwissProt with 6JK8 as a template. Asiatic acid was downloaded from the PubChem NCBI database (CID 119034). Molegro Virtual Docker ver.5 software was used to predict the active site of the IGF-1R with 5 van der Waals parameters. Molecular docking was carried out by Molegro Virtual Docker ver.5 with Grid X96.16; Y107.61; Z157.85; radius 11. Docking validation insulin as a native ligand and IGF-1R were set as root-mean-square deviation (RMSD) <2.0 Armstrong (Å) (Zhang *et al.*, 2020).

Data analysis

The body length and head length data were analyzed by two-way analysis of variance ($p < 0.05$) followed by *post hoc* comparisons performed by Tukey's test. Statistical analysis was performed using the IBM Statistical Program Service Solution version 18 program.

Ethical approval

This research was approved by the Health Research Ethics Committee of Universitas Brawijaya with reference number 92/EC/KEPK-PPDS/03/2019.

Results

Zebrafish embryos are used in animal models of IH. Modifying ambient oxygen tension and observing phenotypic changes in real time is simply because of the zebrafish embryo's rapid development and transparent performance. Additionally, critical components of the hypoxia factor system and their genes have been found in zebrafish that are highly comparable to humans and mammals (Kamei and Duan, 2018).

Effect of CA extract on the body length of zebrafish embryos under IH

The study results of the average body length of zebrafish embryos using the intermittent intrauterine hypoxia model and administration of CA ethanol extract at various concentrations at 3, 6, and 9 dpf (Fig. 1). The results showed that there was a significant difference in body length between the control group and the treatment group at 3, 6, and 9 dpf ($p < 0.05$). There was a significant difference in body length ($p < 0.05$) between the mean (mm) of the IH group (0.50 ± 0.04), IHCA1 (0.51 ± 0.01), IHCA2 (0.50 ± 0.02), IHCA3 (0.48 ± 0.03), and group C (0.65 ± 0.05) at 3 dpf. Group C showed a longer body length than the IH-induced group. The group given CA therapy showed a significant increase in embryo body length compared with the group not given CA. Zebrafish embryos aged 6 and 9 dpf showed an increase in body length compared with those aged 3 dpf in all treatment groups. The study also showed that the body length in the IHCA1 group (0.84 ± 0.05), IHCA2 (0.84 ± 0.07), IHCA3 (0.85 ± 0.07), and C (0.89 ± 0.08) differed significantly from that in the IH group (0.78 ± 0.07) at 6 dpf. Group IH (0.84 ± 0.05), IHCA1 (0.90 ± 0.07), IHCA2 (0.87 ± 0.05), and IHCA3 (0.88 ± 0.07) differed significantly from group C (0.90 ± 0.07) at 9 dpf. There was no significant difference in body length between the IHCA1, IHCA2, and IHCA3 groups with C at 6 and 9 dpf ($p > 0.05$), but IHCA1 tended to have higher body length than the other CA extract treatment groups. The group of zebrafish embryos given CA showed faster body length gain than those not given CA in the IH animal model.

Effect of CA extract on the head length of zebrafish embryos with IH

The results of the mean head length of zebrafish larvae using the intermittent intrauterine hypoxia model and administration of CA ethanol extract at various

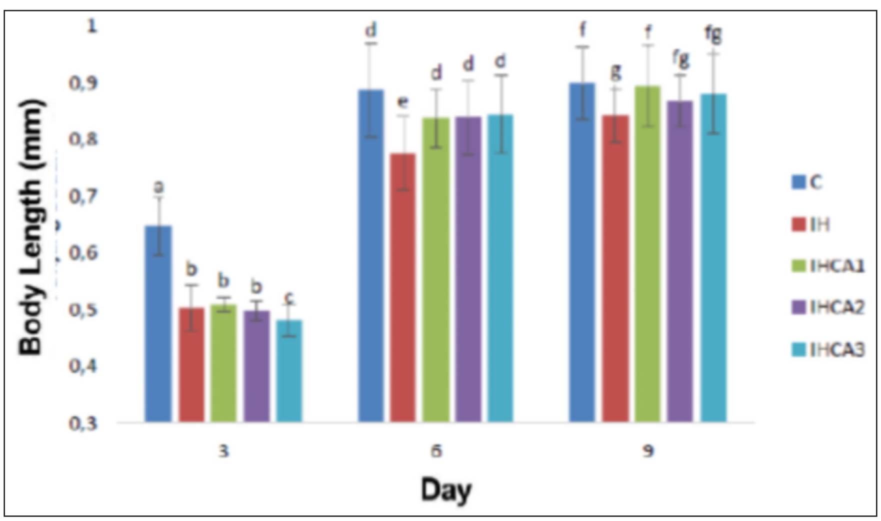

Fig. 1. The body length of embryonic zebrafish at 3, 6, and 9 dpf in the treatment groups. (a–g) Different notations show significant differences. Data are shown as the mean ± SD.

concentrations at 3, 6, and 9 dpf (Fig. 2). The study results showed a significant difference in head length between group C and the IH-induced treatment group at 3, 6, and 9 dpf. There was a significant difference in head length (mm) between group C (0.09 ± 0.01) and group IH (0.07 ± 0.01), IHCA1 (0.07 ± 0.00), IHCA2 (0.07 ± 0.00), and IHCA3 (0.07 ± 0.00) at 3 dpf. In the IH-induced group, there was no significant difference between those given CA extract (IHCA) and those not given CA therapy at 3 dpf. Significant differences in CA concentration began to appear at 6 and 9 dpf. The two treatment groups, group C (0.12 ± 0.01) and IHCA1 (0.11 ± 0.01), did not show a significant difference in head length, but those groups were significantly different from the IH group (0.10 ± 0.01), IHCA2 (0.10 ± 0.02), and IHCA3 (0.11 ± 0.01) at 6 dpf. The C group (0.13 ± 0.01), IHCA1 (0.12 ± 0.01), and IHCA3 (0.12 ± 0.01) had significantly different head lengths than the IH (0.11 ± 0.01) and IHCA2 (0.11 ± 0.01) groups at 9 dpf. The IHCA1 group had a higher average head length than the IHCA2 and IHCA3 groups and was almost the same as the C group, while the IHCA2 group had a shorter head length trend than the other two treatment groups and was the same as the HI group at 6 and 9 dpf.

Molecular interaction prediction of asiatic acid with IGF-1R

Three-dimensional molecular docking prediction showed interaction binding between asiatic acid and the IGF-1R. The results showed interacting residues of the ligand and protein, mediated by five conventional hydrogen bonds and two hydrophobic bonds between amino residues Asn717, Asp328, Val326, Asn349, Val326, and Ala710, but interacting residues of the insulin and IGF-1R, mediated by all eight conventional hydrogen bonds at amino residues Arg351, Asp328, Asn349, and Val326 (Fig. 3). Those bonds interacted with van der Waals energy, thus resulting in an energy binding of asiatic acid to IGF-1R (191.2 kJ/mol) lower than insulin to IGF-1R (−7.2 kJ/mol) (Table 1).

Discussion

Effect of IH in zebrafish embryos

Hypoxia in this animal model was applied as an intermittent schedule at 2, 24, 48, and 72 hpf. At 18 hpf, zebrafish embryos are very tolerant of anoxia, and the cardiovascular and nervous systems are just starting to form. At 24 hpf, the heart tube expands, and the heartbeat starts. At this age, anoxia tolerance decreases, and the embryo upregulates 148 genes in response to severe hypoxia. At 36 hpf, the cardiovascular system is well developed with circulating red blood cells, reduced anoxia tolerance, and embryos hatch into larvae up to 48 hpf (Robertson *et al.*, 2014). In this study, the effect of IH was growth retardation in the body and head of zebrafish, but no inflammation was detected. IH, defined as recurrent hypoxia-reoxygenation cycles, induces inflammation cascades that impact multisystem morbidity, such as retinopathy of prematurity, growth retardation, defects in neuronal development, and the respiratory-circulation system). Many adverse effects of IH on children's behavior, academic achievement, and cognition correlate with hypoxia duration, severity, and onset age. Cycles of hypoxia/reoxygenation frequently lead to a proinflammatory cascade with resulting multisystem morbidity, such as retinopathy prematurity, growth retardation, cardiorespiratory instability, and neurological defects (Serebrovskaya and Xi, 2015). In this study, zebrafish embryos were exposed to IH for 4 hours daily, although it could occur nocturnally. According to Fitzgerald *et al.* (2016), in a seasonal

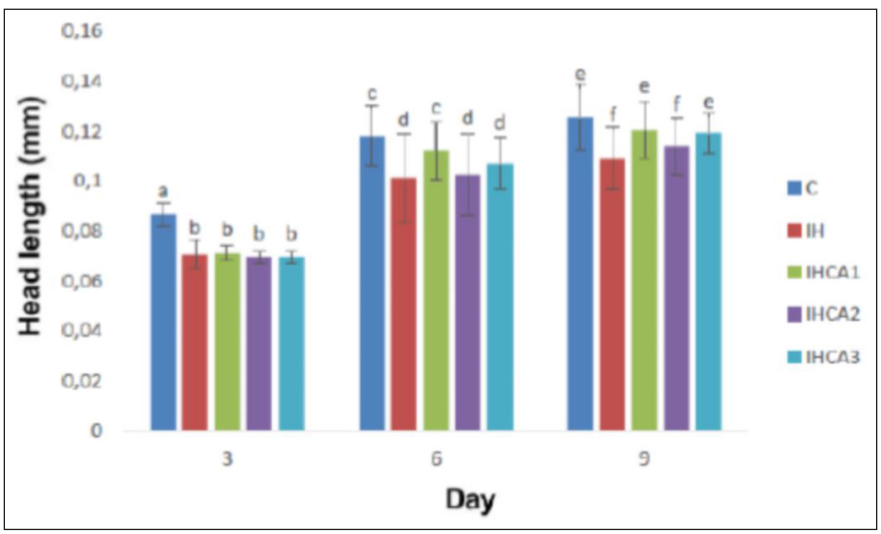

Fig. 2. The head length of embryonic zebrafish at 3, 6, and 9 dpf in the treatment groups. The embryos in the control group were shown to have a significantly more extended head length than those in the other groups ($p < 0.05$). (a–e) Different notations show significant differences. Data are shown as the mean ± SD.

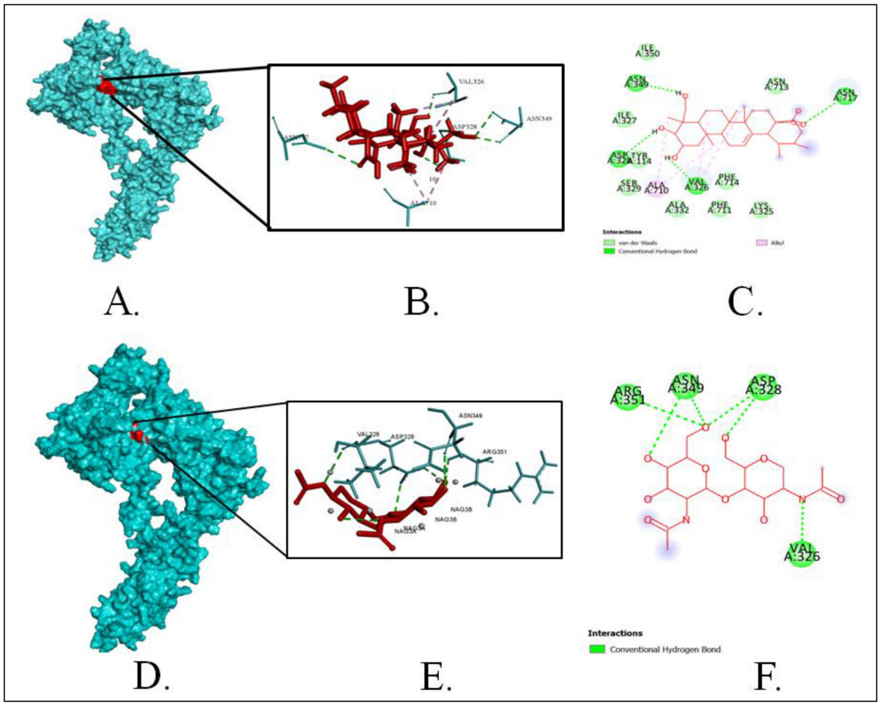

Fig. 3. (A–C) The molecular interaction prediction between asiatic acid from CA and the IGF-1R compared to the IGF-1-insulin complex (D–F).

Table 1. The binding interaction between asiatic acid and the IGF-1R.

Complex	Binding energy (kJ/mol)	Interaction	Distance (Å)	Category	Types
Asiatic acid—IGF1R	191.2	A:ASN717:ND2 - :10:O5	2.86	Hydrogen bond	Conventional hydrogen bond
		10:H42 - A:ASP328:OD2	2.80	Hydrogen bond	Conventional hydrogen bond
		10:H43 - A:VAL326:O	2.10	Hydrogen bond	Conventional hydrogen bond
		10:H47 - A:ASN349:O	2.32	Hydrogen bond	Conventional hydrogen bond
		10:H47 - A:ASN349:OD1	2.70	Hydrogen bond	Conventional hydrogen bond
		A:VAL326 - :10	4.15	Hydrophobic	Alkyl
		A:ALA710 - :10:C26	4.10	Hydrophobic	Alkyl
Insulin—IGF1R	−7.2	A:ARG351:HN - :NAG3B:O6	2.98	Hydrogen bond	Conventional hydrogen bond
		NAG3B:N2:NAG3A:O3	2.97	Hydrogen bond	Conventional hydrogen bond
		_:NAG3B:O6 - A:ASP328:OD1	2.86	Hydrogen bond	Conventional hydrogen bond
		_:NAG3B:O6 - A:ASN349:O	2.69	Hydrogen bond	Conventional hydrogen bond
		_:NAG3B:O4 - A:ASN349:O	2.89	Hydrogen bond	Conventional hydrogen bond
		_:NAG3A:N2 - A:VAL326:O	3.11	Hydrogen bond	Conventional hydrogen bond
		_:NAG3A:O6 - A:ASP328:OD2	2.94	Hydrogen bond	Conventional hydrogen bond
		_:NAG3A:O6 - _:NAG3B:O5	2.76	Hydrogen bond	Conventional hydrogen bond

environment, during the warm season, hypoxia is related to the formation of a thermocline in surface waters, which results in excess oxygen consumption as organic matter decomposes in the lower water layers. Continuous exposure to hypoxia in the process of embryogenesis causes hypoxic events to persist in the long term.

Effect of CA extract on body length and head length of zebrafish embryos with IH

This study found an increase in zebrafish embryo body length and head length following the combination of IH and CA at 3, 6, and 9 dpf. IH causes a reduction in body length and head length. Abnormal embryogenesis was reported on oxygen deprivation of zebrafish models by activation of hypoxia-inducible factor proteins. Oxygen is central to fetal growth and development and adult physiological mechanisms. Therefore, low PO_2 concentration (hypoxia) affects growth, development, organogenesis, and resulting in growth restriction in human fetuses, even in other vertebrate species such as fish (Fathollahipour *et al.*, 2018).

In this study, measurements of body length and head length after administration of CA extract began when zebrafish embryos were at 3 dpf. Zebrafish aged 3–48 hpf elicited greater morphological effects because of the high variability of hormone receptor expression that appeared earlier, which has the potential to bind maternally inherited hormones and allow them to have responsive abilities early in development. Differentiation of the first endocrine gland occurs at 3 dpf (Kinch *et al.*, 2016). According to Lantz-McPeak *et al.* (2015), the drug can spread through the skin and gills of the embryo and enter the body orally when the zebrafish begins to ingest at approximately 72 hpf.

However, this study showed that the mean body length was longer in the IHCA1 group, but the head length was longer in the IHCA2 group than in the other IHCA groups at 6 and 9 dpf. Giving CA extract at 2.5–5 µg/ml is an effective dose to increase the growth of zebrafish embryos under conditions of IH. The results of this study are the same as those conducted by Wardani *et al*. (2018), which showed that CA extracts at a dose of 2.5 could inhibit stunting and improve body length by increasing the expression of vascular endothelial growth factor (VEGF) and VEGF receptor-2 in the stunting model of zebrafish larvae. The increase in body length due to CA treatment was demonstrated by a previous report using rotenone-induced zebrafish larvae by reducing malondialdehyde and elevating superoxide dismutase and catalase as endogenous enzymes. The reduction in oxidative stress was proposed as an underlying pathway following CA treatment in the rotenone-induced maldevelopment zebrafish model (Darwitri *et al.*, 2018). Asiatic acid is an aglycone of asiaticoside, which is a biomarker component and is an indicator of the quality of CA plant extracts because of its high content compared to other components (Gray *et al.*, 2018; Mioc *et al.*, 2022). Treatment with an adequate dose of CA could also reduce reactive oxidative stress (ROS) generation via the enhanced activity of mitochondrial complex I, including mitochondrial DNA, to electron transport of adenosine triphosphate production, which can eventually lead to apoptosis (Lu *et al.*, 2018).

Molecular interaction prediction of asiatic acid with IGF-1R

The results of validation of insulin as a native ligand with the IGF-1R show an RMSD value of ≤2 Å. RMSD shows how much the protein-ligand interaction changes during docking to determine the deviation value. The docking method is valid if the RMSD value ≤2 Å, so the docking method can be used for docking compounds (Utami *et al.*, 2022). In this study, an *in silico* test was also carried out to predict the bond between asiatic acid and IGF-1R. The hydrogen bonding of acetic acid and insulin is maintained to bind with Asn349.

It was observed at a distance of over 2 A. The distance from hydrogen bonds and the free energy of both of them exceeded the simulation time, which is very important to explain the potential compound affinity (Frimayanti *et al.*, 2021). The most important bioactive compounds of CA are triterpene glycosides (saponins), such as asiaticoside, madecassosisi, asiatic acid, and madecassic acid. Asiatic acid exhibits antioxidant, anti-inflammatory, and antiapoptotic effects by reducing ROS production and caspase activity. CA promotes vascular growth by elevating VEGF expression. Signaling via IGF-1R in normal cells leads to its activation of several intracellular pathways mediated by receptor domain-associated tyrosine kinase, phosphatidylinositol 3-kinase, and serine/threonine kinase (Akt), which plays a role in increasing growth and survival (Soni *et al.*, 2015). In this study, hydrophobic and hydrogen bonds played important roles in the interaction between asiatic acid and IGF-1. This study is in line with that of Susanto *et al.* (2018), who found that theaflavin 3,3'-Togliatti has strong binding activity with IGF-1R signaling via hydrogen bonds as an anti-obesity agent by inhibiting cell proliferation (apoptosis agonist). The modulation of the IGF-1 pathway was proposed as a molecular mechanism for predicting pathways. Over five conventional hydrogen bonds, fulfilling one of the criteria of the rule-of-five in drug-likeliness scores of ligands, namely, having five or fewer hydrogen bond donor sites. This study showed different residues from research conducted by Soni *et al.* (2015) in that the Glu1080, Glu1027, Glu1025, Glu1145, Met1028, Arg1003, Arg1064, Arg1029, Lys1150, Leu1081, and Thr1028 residues were found to be significant binding site residues in the IGF-1R protein in various human malignancies.

In fish, the appearance of morpho anatomical deformities is not only influenced by oxygen tension conditions but also related to temperature (Mugwanya *et al.*, 2022). In this study, IH zebrafish embryos that were given CA therapy also exhibited an increase in locomotor activity (zebrafish velocity) at the age of 6.9 dpf (unpublished data). The results of this study follow research conducted by Oliva *et al.* (2021), and there is a correlation between morphological changes and locomotor defects. Thus, acetic acid in CA may serve as an efficient support therapy of IH on IGF-1R targets. Asiatic acid-induced improvement in growth retardation in the concentration-dependent dose. Asiatic acid has a similar interaction with insulin on the active site of the IGF-1R against intermediate hypoxia mediated by the same conventional hydrogen bonds at amino residues Asp328, Asn349, and Val326, although the energy binding of asiatic acid to IGF-1R is lower than that of insulin. A more negative binding free energy means stronger binding (Frimayanti *et al.*, 2021). This indicates that CA activates the insulin signaling pathway as an agonist interaction.

This study shows that changes in the head and body embryo length can go through alternative pathways, such as IGF-1R. This research is in line with Kinch *et al.* (2016), who stated that changes in head morphology, embryo length, pericardial morphology, or egg yolk morphology in zebrafish do not occur through activation of a single hormone receptor pathway but can occur through alternative pathways, such as estrogen-related receptors or IGF-1R hormone receptors. Growth, and glucocorticoid receptors, as components of the growth axis and endocrine stress expressed in the embryonic phase. Further studies need to research other roles of asiatic acid compounds in other receptor signaling molecules as therapeutic targets of IH and observe the criteria of the rule-of-five in the drug-likeliness scores of ligands so that they can be developed as natural drugs.

Conclusion

The hypoxic state of pregnancy is highly implicated in fetal programming. Hypoxia induces oxidative stress and, thus, requires the administration of antioxidants. The administration of CA extract benefits IH through the development and growth of zebrafish embryos at a dose of 2.5–5 µg/ml. There is a binding affinity of asiatic acid to the IGF-1R that plays a role in the IH animal model. Further studies need to research other roles of asiatic acid compounds in other receptor signaling molecules as therapeutic targets of IH and observe the criteria of the rule-of-five in the drug-likeliness scores of ligands so that they can be developed as natural drugs.

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Conflicts of interest

There are no conflicts of interest among the authors in this research and publication.

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Author contributions

AR, HK, NU, MA: concept and design of the research; AR, IF: acquisition of data, analysis, and interpretation; critical revision and final approval: AR, HK, NU, MA.

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