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Original Research Article

Teratogenic potential of ginger (*Zingiber officinale*) extract using chicken (*Gallus gallus domesticus*) embryo development model

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ABSTRACT

Background: Ginger (*Zingiber officinale*) is widely used as a food and folk remedy, including during pregnancy, and is often presumed safe, yet evidence on its developmental toxicity is limited. This study evaluated the teratogenic potential of ginger extract using the chicken (*Gallus gallus domesticus*) embryo model.
Methods: This experimental study was conducted at the Philippine Science High School Ilocos Region Campus, Ilocos Sur, from November to December 2024. Seventy-two fertilized native chicken eggs were allocated to six groups (n=12) with four ginger extract concentrations (0.01, 0.1, 0.25, 0.5 mg/ml), saline (negative control), and retinol (positive control). Each received a single 0.1 ml injection on day 3 of incubation, and eggs were opened on days 12, 15, 18, and 21 to assess mortality, heart rate, weight, and crown-rump length (CRL). Data were analyzed by MANOVA with LSD post hoc tests.
Results: Mortality was 0% at 0.01 mg/ml and in the negative control, 16.66% at 0.1 mg/ml, and 33.33% at 0.25 mg/ml, 0.5 mg/ml, and in the positive control. Weight differed significantly among groups on day 15 (p=0.039) and day 21 (p=0.021); the multivariate effect was significant only on day 21 (p=0.002). Heart rate and CRL showed non-significant dose-related trends.
Conclusions: Higher ginger extract concentrations were associated with greater embryonic mortality and altered weight, whereas the lowest concentration had no observable effect. Ginger is not established as a teratogen, and findings cannot be extrapolated directly to human intake, larger studies are warranted.

Keywords: Teratogenicity, Herbal medicine, Ginger extract, *Zingiber officinale*, Chicken embryo model, Embryotoxicity

INTRODUCTION

Herbal remedies have been used in traditional medicine for centuries. Before the advent of synthetic pharmaceuticals, communities relied on the bark, roots, bulbs, fruits, and leaves of local plants to treat conditions ranging from wounds to parasitic infections.[1,2] These preparations have since expanded into daily beverages, culinary ingredients, and over-the-counter supplements, and the belief underlying their use has changed little, that a remedy derived from plants is inherently mild and safe.

This belief is not supported by examination and evidence. Plant tannins, for example, are antimicrobial against diarrheal pathogens, yet they also bind digestive enzymes and proteins and thereby impair nutrient absorption.[3] Other herbal preparations have been linked to kidney injury, liver damage, and neurotoxicity. Moreover, many communities rely almost entirely on traditional healers for guidance on

medicinal plants, with limited independent verification of safety.[1] The resulting picture is one of widespread use supported by thin evidence, which is of particular concern when the users include pregnant women.

Ginger occupies a distinctive position among these remedies. It is at once a spice, a folk medicine, and, increasingly, a subject of formal pharmacological investigation, most notably for its effectiveness against nausea and vomiting in early pregnancy. However, its long history of casual use has seldom been matched by rigorous developmental toxicology. This study was therefore designed to address this gap, using the developing chicken embryo, an established model of teratogenic response, to determine whether ginger extract poses a developmental risk and at which concentrations such a risk becomes apparent. The aim is not to discredit a widely trusted remedy but to subject it to the same scrutiny required of any substance before it is recommended during pregnancy.

Statement of the problem

This study aimed to determine the teratogenic potential of *Zingiber officinale* (ginger) as a conventionally used health remedy, using the developing chicken embryo as a model system relevant to patterns of human teratogenicity. Specifically, it sought to answer: 1. What are the effects of *Zingiber officinale* extract on chick embryo development, measured in terms of mortality rate, heart rate, weight, and crown-rump length? 2. Do these effects differ significantly across treatment concentrations, for each of the four parameters listed above?

Related literature

Ginger and teratogenicity

Zingiber officinale, known locally as luya, is a rhizomatous, monocot plant native to shaded, moisture-rich tropical environments, particularly in Southeast Asia.[4] Its rhizome contains a dense array of bioactive compounds, gingerdiol, gingerdione, gingerol, and shogaols, alongside iron, magnesium, calcium, vitamin C, and flavonoids, giving it broad anti-inflammatory, antioxidant, analgesic, and anti-apoptotic properties.[5,6] In the Philippines specifically, ginger decoctions are used for stomach ache, topical applications for headache, and pounded rhizome for arthritic joints, reflecting a depth of ethnomedicinal use that long predates any formal safety testing.[4]

The presumption that herbal medicines are inherently mild rests on precarious ground, since the same phytochemical complexity that gives plants their therapeutic range can also produce interactions that amplify rather than neutralize harm.[7] Ginger's two principal active compounds, gingerol and shogaol, are responsible for much of its therapeutic reputation, as gingerol contributes antiemetic activity, while shogaol acts on serotonin receptors in the gut, a mechanism that underlies its popular use against morning sickness.[8,9]

The literature on its developmental safety, however, is inconsistent. A standardized ethanol extract of ginger was found to be neither embryotoxic nor teratogenic in rats and was well tolerated.[10] By contrast, ginger tea given to pregnant rats was associated with increased embryonic loss.[11] In humans, dried ginger did not appear to act as a major teratogen, although the same study noted a marginal increase in stillbirth risk among women who used it for various ailments during pregnancy.[12] In addition, a methanolic ginger extract showed anti-angiogenic activity in a chorioallantoic membrane assay, reducing blood vessel diameter.[13] This finding is relevant as anti-angiogenic activity has been mechanistically linked to teratogenicity; the developmental effects of thalidomide, for instance, are attributed to suppression of nascent blood vessel growth.[14] Ginger's anti-angiogenic properties may therefore carry a similar risk under certain conditions. Much of the inconsistency among studies probably arises from differences in the chemical composition of the extracts tested, a caveat that also applies to present study.[7]

Gallus gallus domesticus

Native chickens are a familiar presence in Philippine households, and genetic studies trace them closely to the red jungle fowl.[15] Beyond their culinary and cultural roles, chickens have long served scientific inquiry, and the developing chick embryo in particular has become a standard model for teratogenicity research. Researchers point out that much of what is understood about vertebrate embryological development originated from chick studies, and the model offers practical advantages besides, the embryo can be observed continuously across gestation, test compounds are simple to administer directly into the egg, and the full incubation period runs only 20 to 21 days, considerably shorter than comparable avian species such as duck or turkey.[16-18]

Retinol and teratogenicity

Retinol and its derivatives are among the most thoroughly documented human teratogens, which is precisely why the compound was selected as this study's positive control. During embryogenesis, vitamin A interferes with neural-crest cell migration, producing a recognizable pattern of craniofacial, cardiac, and thymic defects known as retinoic acid embryopathy. Topical formulations appear comparatively low-risk given their limited systemic absorption, but the compound's teratogenic profile at systemic or embryonic-injection doses is well established.[19] There are documented cranial and brain size reduction, forelimb malformation, and cardiomegaly with inhibited cardiomyocyte development in chick embryos exposed to excess retinoic acid, while others found that retinoic acid exposure significantly reduced embryo survivability, a result this study's own positive control data corroborate.[20,21]

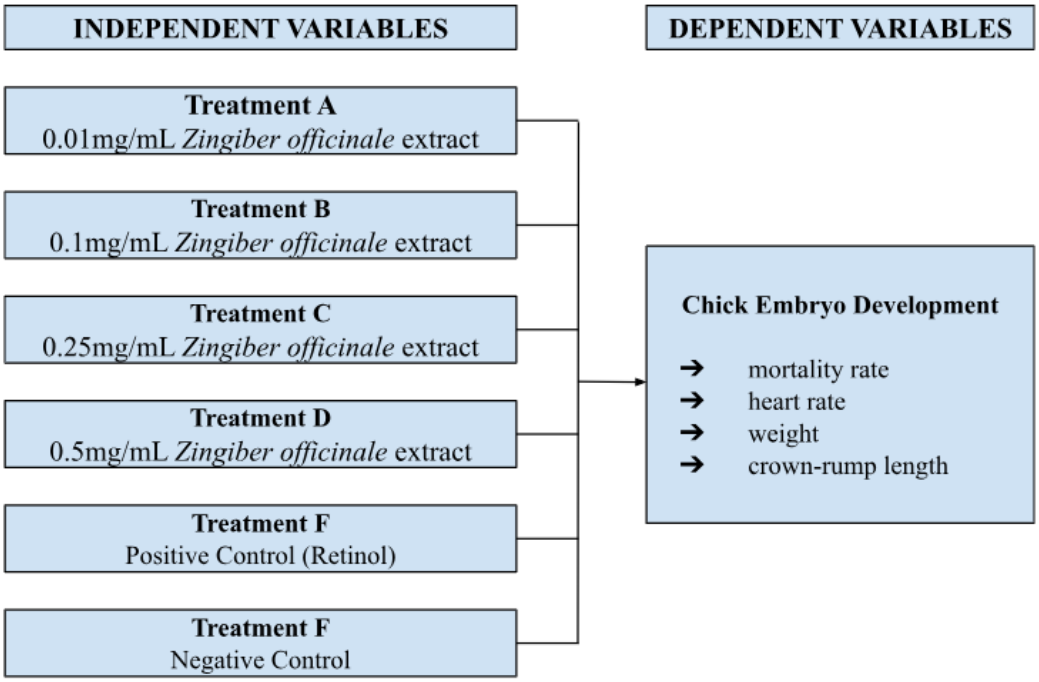


Figure 1: Research paradigm showing the treatment groups (independent variables) and the embryonic parameters measured (dependent variables including mortality, heart rate, weight, and CRL).

METHODS

Study type and design

This was an experimental laboratory study that used a completely randomized design (CRD) to evaluate the effects of ginger extract on chick embryo development. In experimental research, the investigator manipulates the independent variable, which in this study was the concentration of ginger extract administered at a fixed volume.[22] Under a CRD, each fertilized egg had an equal probability of being assigned to any treatment group, which limits the influence of pre-existing differences between eggs on the outcomes observed.[23]

Seventy-two fertilized eggs were divided into six groups of twelve: four ginger extract concentrations (0.01, 0.1, 0.25, and 0.5 mg/ml; Treatments A to D, respectively), one positive control (retinol), and one negative control (normal saline). Within each group, three eggs were assigned to each of four opening days (days 12, 15, 18, and 21 of incubation), giving three embryos per group at each timepoint (n=18 embryos per timepoint across the six groups). Because the eggs were opened for assessment, this cross-sectional structure means that embryos assessed on day 21 were not the same individuals measured on day 12; each opening day represents an independent subset of eggs from the same treatment group.

Study place and period

The study was conducted at the Philippine Science High School Ilocos Region Campus, San Ildefonso, Ilocos Sur, Philippines, November to December 2024. The incubator was maintained under controlled laboratory conditions to minimize extraneous stressors such as excess moisture, vibration, and pest exposure. Ginger rhizomes were sourced from a local farm in Tagudin, Ilocos Sur, and native chicken eggs were procured from a poultry supplier in the Municipality of Narvacan, Ilocos Sur.

Selection criteria of the study sample

Fertilized native chicken (*Gallus gallus domesticus*) eggs was obtained from a single poultry supplier. Eggs with intact, uncracked shells were eligible, whereas cracked or visibly contaminated eggs were excluded. Fresh eggs are selected of similar size and weight that had not been refrigerated or washed, and eggs showing viable embryonic development on candling before treatment. A total of 72 eggs were used and allocated equally to the six treatment groups (12 eggs per group). One locally cultivated ginger variety was used as the plant material.

Procedure

Materials

The materials used included a thermostat-regulated incubator, a food processor for pulverizing dried ginger, beakers for extract storage, syringes for injection, paraffin film for sealing injection sites, alcohol and cotton for surface sterilization, a lancet for boring injection holes, normal saline solution (NSS) for dilution, an analytical balance for weighing embryos, a dissecting microscope fitted with a measuring scale for CRL assessment, standard

recording materials, and designated containers for biological waste disposal.

Preparation of the methanolic extract

Fresh ginger rhizomes were washed, peeled, sliced, and oven-dried, then pulverized and macerated in 80% methanol for 72 hours at room temperature. The mixture was filtered to separate the solid residue, and the filtrate was concentrated by evaporating the methanol using a rotary evaporator. The concentrated crude extract was stored at 4°C until use.

Preparation of treatment solutions

The stock extract was serially diluted in NSS to obtain working concentrations of 0.01, 0.1, 0.25, and 0.5 mg/ml, following a dilution scheme based on a published methodology.[24] These concentrations were selected with reference to a reported plasma Cmax of approximately 4.23 µg/ml for [6]-gingerol after oral administration; the higher mg/ml range used here reflects the crude, unpurified nature of the extract, in which [6]-gingerol represents only a fraction of the total bioactive content, and was not intended to replicate plasma concentrations directly.[25] The negative control consisted of NSS alone, and the positive control consisted of retinol soft gel capsules.

Administration of treatments

Egg surfaces were sterilized with 70% alcohol before treatment. A lancet was used to bore a 0.3 mm hole above the air sac, through which 0.1 ml of the assigned solution was injected using a 1 cc syringe on the third day of incubation, corresponding to Hamburger-Hamilton stage 14.[26] The injection site was then sealed with paraffin film to prevent contamination, and the eggs were returned to the incubator until their designated opening day.

Incubation and daily observation

Eggs were checked daily for visible changes in shell color, odor, or early hatching, and all observations were logged by date.

Assessment of outcome parameters

Eggs were opened on days 12, 15, 18, and 21 of incubation, and embryos were examined using a dissecting microscope fitted with a measuring scale. Mortality was recorded by direct visual inspection and manual count. Heart rate was measured in beats per minute over a full one-minute interval. Weight was recorded using an analytical balance, and CRL was measured using the microscope's calibrated scale. Any visible malformations were documented and cross-referenced against treatment concentration to identify potential dose-related patterns. Where feasible, the researchers recording heart rate, weight, and CRL were not informed of the treatment group at the time of measurement, to reduce observer bias.

Disposal of materials

All laboratory equipment was cleaned and disinfected after use. Non-reusable materials, including eggshells, were discarded appropriately. Non-viable embryos were wrapped in aluminum foil, sealed in a container, and buried at a depth of four feet in an unoccupied area free of nearby households or livestock, in keeping with standard practice for the disposal of biological specimens.

Ethical approval

Ethical clearance was obtained from the Ethics Review Committee of the University of Northern Philippines before the start of the experiment. Because chick embryos may begin to develop nociceptive responses in the later stages of incubation, all opening and assessment procedures were performed promptly and with care to minimize distress, and no embryo was subjected to any procedure beyond what was necessary for the measurements described.

Statistical analysis

Descriptive statistics (means and standard deviations) were computed for heart rate, weight, and CRL at each opening day. Mortality was expressed as the proportion of dead embryos per group and was summarized descriptively without inferential testing. Differences among treatment groups were tested using multivariate analysis of variance (MANOVA), with post hoc pairwise comparisons by the Least Significant Difference (LSD) test where the overall MANOVA or the univariate test reached significance. All analyses were performed using SPSS, with the level of significance set at $\alpha=0.05$.

Because each opening day represents a distinct, independently sampled subset of embryos rather than repeated measurements on the same individuals, a separate MANOVA was run for each timepoint instead of a single repeated-measures model. This approach suits the destructive nature of the sampling, but the result for each day should be read as a snapshot of a different embryo cohort rather than as a trajectory followed over time. With only three embryos per treatment group at each timepoint, statistical power and the reliability of variance estimates are limited, and this should be weighed when interpreting the non-significant findings.

RESULTS

Effects of ginger extract on chick embryo development

Mortality rate

Mortality increased with ginger concentration (Table 1). No deaths occurred in the 0.01 mg/ml group (Treatment

A) or the negative control, whereas the 0.1 mg/ml group (Treatment B) had the 16.66 percent mortality (2 of 12 eggs).

The 0.25 and 0.5 mg/ml groups (Treatments C and D) each reached 33.33% mortality (4 of 12 eggs), equal to the rate in the positive control group.

Heart rate

Heart rate varied considerably across treatment groups and opening days (Table 2). At every timepoint, the negative control and 0.01 mg/ml groups had mean heart rates of approximately 212 to 253 beats per minute with small standard deviations.

In contrast, the 0.25 mg/ml, 0.5 mg/ml, and positive control groups had lower means (143 to 165 beats per minute) with very large standard deviations, often approaching the mean itself; the 0.1 mg/ml group showed the same pattern on days 15 and 18.

This pattern reflects the inclusion of non-viable embryos (scored as zero) together with viable embryos in the same small groups, rather than a uniform reduction in heart rate among surviving embryos.

Weight

Mean weights of the 0.25 mg/ml, 0.5 mg/ml, and positive control groups remained within a narrow range across opening days (approximately 45.5 to 50.9 g), whereas the 0.01 mg/ml group had the lowest means (37.33 to 41.76 g) (Table 3). The negative control peaked on day 15 (49.97 g) and declined to 43.57 g on day 21. By day 21, the 0.5 mg/ml and positive control groups recorded the highest mean weights (49.25 and 49.28 g, respectively).

CRL

Mean CRL increased over the incubation period in the negative control and in the 0.01 and 0.1 mg/ml groups, reaching 8.25, 8.33, and 8.58 cm, respectively, on day 21 (Table 4). In contrast, the 0.25 mg/ml, 0.5 mg/ml, and positive control groups had shorter and more variable values on days 18 and 21 (5.37 to 6.28 cm). The 0.1 mg/ml group showed similarly low and variable values on days 15 and 18 (3.98 and 5.92 cm).

Statistical significance of treatment effects

The results of the multivariate and univariate tests for each opening day are summarized in Tables 5 and 6.

Table 1: Mortality of fertilized chicken embryos across treatment groups.

Treatments	Fertilized eggs	Dead	Alive	Mortality rate (%)
A-0.01 mg/ml	12	0	12	0.00
B-0.1 mg/ml	12	2	10	16.66
C-0.25 mg/ml	12	4	8	33.33
D-0.5 mg/ml	12	4	8	33.33
Positive control	12	4	8	33.33
Negative control	12	0	12	0.00

Table 2: Heart rate (beats per minute) by treatment group and opening day.

Treatments	Day 12 M	Day 12 SD	Day 15 M	Day 15 SD	Day 18 M	Day 18 SD	Day 21 M	Day 21 SD
0.01 mg/ml	211.67	1.53	237.33	2.52	228.67	1.53	246.67	2.08
0.1 mg/ml	212.33	6.43	158.00	136.84	154.33	133.68	251.67	1.53
0.25 mg/ml	143.33	124.23	162.67	140.89	157.33	136.26	162.33	140.59
0.5 mg/ml	147.33	127.66	157.00	135.97	159.33	137.99	164.00	142.03
Positive control	160.33	138.88	160.00	138.57	162.33	140.59	164.67	142.61
Negative control	214.67	1.15	236.67	2.08	237.33	1.53	253.00	3.00

Table 3: Weight (grams) by treatment group and opening day.

Treatments	Day 12 M	Day 12 SD	Day 15 M	Day 15 SD	Day 18 M	Day 18 SD	Day 21 M	Day 21 SD
0.01 mg/ml	41.76	1.33	37.33	4.31	40.77	0.71	40.32	0.70
0.10 mg/ml	39.63	1.60	44.60	6.76	46.53	6.60	42.42	0.64
0.25 mg/ml	45.52	6.34	46.93	3.70	46.67	5.34	47.32	5.44
0.50 mg/ml	46.36	4.40	50.90	3.33	48.73	3.01	49.25	3.69
Positive control	47.95	5.20	47.90	4.85	48.07	5.14	49.28	4.14
Negative control	41.46	0.79	49.97	3.03	45.60	2.90	43.57	1.29

Table 4: CRL (cm) by treatment group and opening day.

Treatments	Day 12 M	Day 12 SD	Day 15 M	Day 15 SD	Day 18 M	Day 18 SD	Day 21 M	Day 21 SD
0.01 mg/ml	5.67	1.15	5.75	0.25	8.58	0.38	8.33	0.29
0.1 mg/ml	5.42	0.52	3.98	2.87	5.92	4.26	8.58	0.38
0.25 mg/ml	3.50	2.60	4.53	3.42	6.05	4.46	5.37	3.70
0.50 mg/ml	4.08	3.17	4.27	3.00	6.28	4.49	5.88	4.32
Positive control	3.90	2.82	3.98	2.85	5.92	4.26	6.00	4.36
Negative control	5.17	0.29	6.17	0.52	8.33	0.38	8.25	0.25

Table 5: Multivariate tests (MANOVA, Wilks' Λ) of the treatment effect on heart rate, weight, and CRL at each opening day.

Opening day	Wilks' Λ	F	Hypothesis df	Error df	Sig.
Day 12	0.226	1.331	15.000	28.007	0.249
Day 15	0.164	1.730	15.000	28.007	0.102
Day 18	0.203	1.457	15.000	28.007	0.189
Day 21	0.056	3.422	15.000	28.007	0.002

*Intercept effects are omitted.

Table 6: Univariate between-subjects effects of treatment on each parameter at each opening day (F and p values; df=5, 12).

Parameters	Day 12 F	Day 12 p	Day 15 F	Day 15 p	Day 18 F	Day 18 p	Day 21 F	Day 21 p
Heart rate	0.425	0.822	0.380	0.853	0.359	0.867	0.676	0.650
Weight	2.112	0.134	3.367	0.039	1.238	0.351	4.094	0.021
CRL	0.558	0.730	0.430	0.819	0.371	0.859	0.749	0.602

Day 12

The overall MANOVA showed no significant treatment effect (Wilks' Λ=0.226, F(15, 28)=1.331, p=0.249). None of the three parameters (heart rate, weight, or CRL) reached significance individually, and post hoc comparisons revealed the no significant pairwise differences.

Day 15

The overall MANOVA again fell short of significance (Wilks' Λ=0.164, F(15, 28)=1.730, p=0.102), but weight alone showed a significant treatment effect (p=0.039). Post hoc comparisons located this difference at the low end of the dose range: the 0.01 mg/ml group, which had the lowest mean weight, differed significantly from the 0.25 mg/mL (p=0.028), 0.5 mg/ml (p=0.004), positive control (p=0.017), and negative control (p=0.006) groups.

Day 18

No significant overall treatment effect was found (Wilks' Λ=0.203, F(15, 28)=1.457, p=0.189), and none of the three parameters reached significance individually. One pairwise post hoc comparison for weight, between the 0.01 as well as 0.5 mg/ml groups, reached the threshold (p=0.047).

Day 21

By day 21, the overall MANOVA reached significance (Wilks' Λ=0.056, F(15, 28)=3.422, p=0.002), the strongest treatment effect observed at any timepoint. Weight again drove this result (p=0.021). Post hoc tests showed significant differences between the 0.01 mg/ml group and each of the 0.25 mg/ml (p=0.021), 0.5 mg/ml (p=0.005), and negative control (p=0.005) groups, as well as between the 0.1 mg/ml group and both the 0.5 mg/ml (p=0.024) and negative control (p=0.024) groups. Across all timepoints, the lighter group in every significant pairwise contrast was the 0.01 mg/ml group or, on day 21, the 0.1 mg/ml group, and no significant difference in weight was found between any group receiving 0.25 mg/ml or more and the negative control. These data indicate that ginger extract concentration was associated with differences in embryonic weight, although this association was not a simple increase with dose relative to the negative control.

DISCUSSION

Mortality increased with ginger concentration, and the two highest ginger concentrations (0.25 and 0.5 mg/ml) produced a mortality rate (33.33%) equal to that of the retinol positive control. The mortality data was not tested inferentially and each group comprised only 12 eggs, this similarity should be interpreted cautiously. The findings do not imply that ginger and retinol act through the same

mechanism, which underlying biological pathway is almost certainly different.

The negative control had among the highest heart rates at all four opening days, whereas the higher-dose and positive control groups had lower means with standard deviations that in several cases equaled or exceeded the mean. As non-viable embryos were scored as zero, this pattern most likely reflects mortality within small groups rather than depression of heart rate in surviving embryos, and the absence of significant differences in heart rate should be read in that light. According to a published report, gingerol at high concentrations disrupts cardiomyocyte differentiation and viability in vitro, which is compatible with cardiac vulnerability, but the present data cannot separate a true cardiotoxic effect from small-sample variability.[27] Larger samples, with heart rate measured only in viable embryos, are needed in any follow-up study.

Weight differed significantly among treatment groups on days 15 and 21, with the higher-dose and positive control groups heavier than the lowest-dose group. Such a difference is unlikely to represent accelerated tissue growth and may instead reflect impaired yolk sac retraction, a recognized contributor to increased weight during late incubation.[28] Yolk provides most of the energy required for embryonic development, with more than 90% of that energy derived from lipid oxidation, and any disruption of the blood flow that supports this process can leave more yolk unabsorbed at the time of measurement.[29,30] If ginger extract at higher concentrations interferes with yolk retraction, whether directly or through its documented anti-angiogenic activity, this would represent a genuine developmental effect rather than statistical noise.[13] However, this explanation is not further supported as yolk sac retraction and vascularization were not measured directly. Moreover, the significant contrasts were confined to comparisons with the lowest-dose groups, and no group receiving 0.25 mg/ml or more differed significantly from the negative control. The weight findings should therefore be regarded as a signal requiring confirmation rather than as evidence of a dose-response relationship.

CRL showed a broadly similar dose-related pattern, being shortest at the higher concentrations and in the positive control and longest in the negative control and lowest-dose groups, but no difference reached statistical significance at any timepoint. Gingerol and shogaol are plausible candidates for interference with the cellular pathways underlying embryonic morphogenesis, and the consistent direction of the CRL data across the four opening days is suggestive.[24] It points toward a possible threshold between 0.1 and 0.25 mg/ml, below which growth was largely undisturbed and above which it trended toward impairment. As with heart rate, zero values assigned to non-viable embryos contributed to the low means and wide standard deviations at the higher concentrations, so growth restriction in surviving embryos cannot be inferred from these data.

The malformations observed in the positive control group, namely crooked and asymmetric limbs, patchy feathering, reduced CRL, and incomplete yolk retraction, are consistent with the established mechanism of retinoic acid embryopathy, in which disrupted neural-crest migration and altered Hox gene expression interfere with limb and skeletal patterning.[20,21,31] These findings mainly confirm that the model behaved as expected, which supports the validity of the ginger-related observations made alongside it.

CONCLUSION

Ginger extract affected chick embryo development in a concentration-dependent but non-uniform manner. Mortality increased with concentration, reaching the level of the retinol, a known teratogen, at two highest concentrations tested. Weight was the only parameter that differed significantly among groups, with higher-dose groups heavier than the lowest-dose group, which may reflect impaired yolk sac retraction rather than somatic growth. Heart rate and CRL showed dose-related trends that did not reach statistical significance.

These findings do not establish ginger extract as a teratogen comparable to retinol at the concentrations tested. These inferences, however, do suggest that at higher concentrations, ginger extract produces measurable, dose-dependent physiological disruption in a developing embryo, and this signal should not be dismissed merely because not every parameter reached significance at every timepoint. Lower concentrations of *Zingiber officinale* appear to leave embryonic development largely undisturbed, while higher concentrations carry, if not yet fully characterized, developmental risk. As the injected concentrations have not been related to human dietary exposure, the results cannot be extrapolated directly to ginger consumption during pregnancy; these support a cautious approach, pending confirmation in larger studies and in models that reflect maternal exposure. Pregnant individuals and the practitioners advising them would be reasonable to treat this as grounds for moderation rather than avoidance.

Limitations

The study was limited to the observation of ginger extract's effects on developing *Gallus gallus domesticus* embryos using seventy-two fertile native chicken eggs sourced from a poultry farm in Narvacan, Ilocos Sur. Eggs were injected with one of four ginger extract concentrations (0.01, 0.1, 0.25, and 0.5 mg/ml), a plain saline negative control, or a retinol positive control. Only one locally cultivated ginger variety was used. Eggs were opened at four incubation timepoints, 12, 15, 18, and 21 days, for assessment of mortality, heart rate, weight, and CRL. Findings are therefore specific to this ginger variety, this dose range,

and this embryonic model, and should not be generalized to other preparations, dosing routes, or species without further study.

The sample was small (three embryos per group at each timepoint), which limited statistical power and the reliability of variance estimates, and the cross-sectional design did not allow individual embryos to be followed over time. Pairwise comparisons used unadjusted LSD tests, which increased the risk of type I error, and scoring non-viable embryos as zero inflated the variance of heart rate and CRL. A crude methanolic extract without phytochemical standardization was used, so the effects cannot be attributed to specific compounds. In addition, a single in ovo injection bypasses maternal absorption, metabolism, and placental transfer, which limits extrapolation to human pregnancy. Finally, the injected concentrations were not converted to human-equivalent dietary doses, so these findings cannot be used to define a safe intake of ginger during pregnancy.

Recommendations

Future work should prioritize a substantially larger number of fertilized eggs per treatment group and per opening day, both to improve statistical power and to reduce the influence of individual embryo variability on group-level estimates. Where feasible, researchers should also consider longitudinal tracking methods that allow the same embryo's development to be followed over time, rather than relying solely on cross-sectional sampling at fixed incubation days.

Additional developmental parameters, vascular development, gross morphological malformation scoring, and gait or stance abnormalities in hatched chicks, would add depth beyond the four parameters examined here. Testing a wider concentration range, including doses beyond 0.5 mg/ml and a purified rather than crude extract, would help clarify whether the effects observed here scale further at higher exposure or plateau. Extending the duration of exposure, rather than a single injection, would also help determine whether cumulative, chronic exposure produces different or more pronounced effects than a single dose.

Given the anti-angiogenic activity documented for ginger extract elsewhere in the literature, future studies might usefully include a direct chorioallantoic membrane angiogenesis assay alongside the standard teratogenicity parameters, to test more directly whether impaired vascularization underlies the weight and mortality effects observed here. Finally, phytochemical profiling of the specific extract used, quantifying gingerol and shogaol content in particular, would allow future researchers to relate observed effects back to specific compounds rather than to the crude extract as a whole, and would support more direct comparison across studies that use different ginger preparations.

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