International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 07 Issue 05 May 2024
DOI: 10.47191/ijcsrr/V7-i5-69, Impact Factor: 7.943
IJCSRR @ 2024
www.ijcsrr.org

Teratogenic Risk Potentiality of Blue Ternate (*Clitoria ternatea*) Leaf Extract Using Chorioallantoic Membrane (CAM) Assay: Phase One

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ABSTRACT: The study implemented a descriptive classic experimental design within a laboratory, utilizing the Chorioallantoic Membrane (CAM) assay to investigate the teratogenic potential of Blue Ternate (*Clitoria ternatea*) leaf extract using the different extract concentrations, and the phytochemical analysis screening unveiled the presence of flavonoids, saponins, tannins, alkaloids, and steroids in the leaves. The primary objectives encompassed the assessment of teratogenicity at various extract concentrations, comparing results with controls, and exploring the plant's impact on embryonic development. While no significant differences emerged in primary and secondary blood vessels among treatments, a conspicuous variation in tertiary blood vessels indicated potential teratogenic effects at specific concentrations. Nevertheless, the study concluded that *Clitoria ternatea* exhibits promising therapeutic attributes. Recommendations include the implementation of public awareness programs elucidating the health benefits of Blue Ternate and further investigations into refining, storage practices, and potential teratogenicity in other plant parts. These insights, of considerable value to medical practitioners and future researchers, underscore the importance of caution and consultation, especially for pregnant individuals. In summary, the research significantly contributes to understanding Blue Ternate's safety profile, presenting avenues for future exploration. The emphasis on careful consideration in medicinal applications, particularly during pregnancy, reinforces the need for responsible and informed decision-making in healthcare practices.

KEYWORDS: Blue ternate, Chorioallantoic Membrane, Experimental, Phytochemical analysis, Surigao, Philippines

INTRODUCTION

World Health Organization (WHO) defines traditional medicine as "health methods, techniques, knowledge, and values that include medicinal products, animals and minerals, religious therapies, manual activities, and exercises used alone or in combination for the management, treatment, and prevention of disease as well as for general well-being."

The effectiveness, efficacy, and safety of herbal medicine practices are often not substantiated by empirical investigations. This lack of scientific evidence gives rise to apprehensions over the potential hazards associated with their use, particularly among vulnerable populations such as pregnant women, who are at a higher risk of teratogenic effects. The utilization of herbal medicines for addressing issues related with pregnancy is prevalent, despite the absence of scientific proof. This can be attributed to the widely held belief that these products are devoid of hazardous effects and adverse reactions, as they are derived from natural sources. The little attention given to the utilization of herbal treatments during pregnancy is reinforced by the absence of comprehensive regulatory frameworks in many countries governing their marketing practices. Nevertheless, it should be noted that plant-based medicines are not exempt from the occurrence of undesirable responses. Medicinal plants and herbal remedies possess bioactive compounds that have the potential to have harmful effects on both the human body and the developing baby (Bernstein, N., 2020).

Currently, *Clitoria ternatea*, also known as Blue Ternate, is a traditional medicinal plant that has gained significant attention and recognition from various researchers. The plant in question is an herbaceous perennial vine that falls within the Fabaceae tribe. The plant is primarily distributed across the tropical regions of India, Sri Lanka, Malaysia, Burma, and the Philippines. (Nileththi, 2019). Moreover, the utilization of *C. ternatea* in traditional medicine has been extensive, particularly as a supplementary treatment to augment cognitive abilities and mitigate various conditions such as fever, inflammation, discomfort, and diabetes (Mukherjee, 2008).

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Despite the widely reported safe pharmaceutical and therapeutical applications of *Clitoria ternatea*, there is no research finding reporting teratogenic effects. Therefore, in this study, the researchers are prompt and motivated to assess the teratogenic risk potentiality of Blue Ternate *(Clitoria ternatea)* leaf extract through the CAM model.

FRAMEWORK

Fetal exposure to teratogens accounts for about 4% to 5% of congenital disorders. Studies have shown that teratogens cause congenital disorders and increase the chance of miscarriage, stillbirth, other pregnancy complications, and cognitive and physical development. The three primary standards of developmental toxicity by teratogens include delayed growth of organ systems, growth retardation, and the most severe congenital disabilities in live offspring. (Yahya, A. L., et al, 2017).

The teratogenic effect produces embryonic defects by inhibiting the growth of naive, newly forming blood vessels (Beedie, S. L., 2016). In this study, the crude ethanolic extract of Blue Ternate (Clitoria Ternatea) is to evaluate possible teratogenic effects on the vascular density, weight of the embryo, and morphometric indices such as the length of the crown-rump, head-beak, forelimb, and hand limb of an 8 day-old duck egg.

RESEARCH OBJECTIVES

This study determined the teratogenic effect of Blue Ternate (*Clitoria ternatea*) leaf extract via chorioallantoic membrane (CAM) assay. Specifically, this study determined:

1. The phytochemical components of the *Clitoria ternatea* leaf.
2. The teratogenic effect of the Blue Ternate (*Clitoria ternatea*) leaf-derived ethanolic extract with different concentrations based on the following setups:
    2.1 Positive Control (Vitamin A)
    2.2 100 um concentration
    2.3 1,000 um concentration
    2.4 10,000 um concentration
    2.5 Negative Control (Distilled Water)
3. The significant difference between the treatment output of the control set-ups and the experimental set-ups.

METHODS

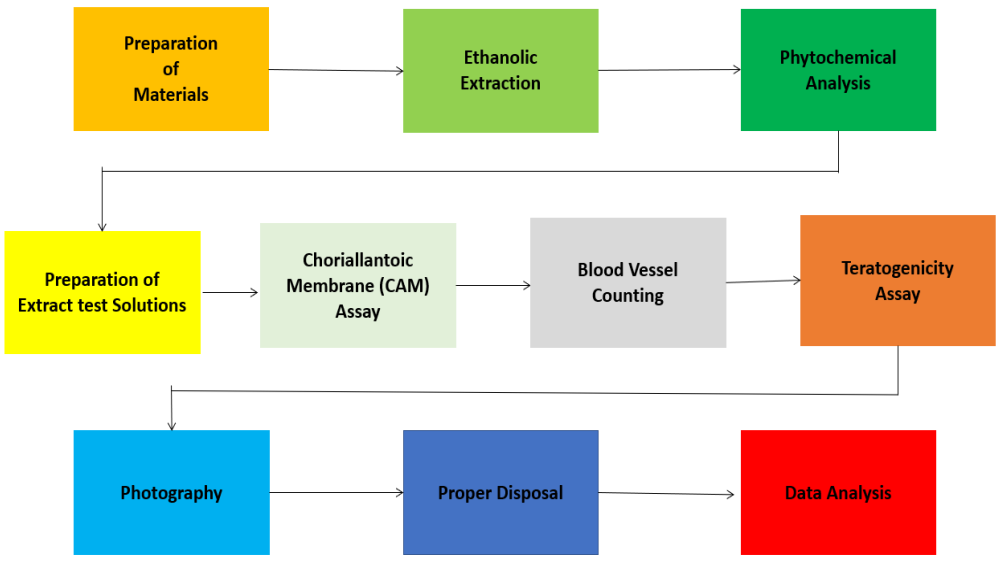

Figure 1. Flowchart of Methodology

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In this study, the researchers employed a quantitative approach following the classic experiment design with data collection done in the laboratory. The CAM assay is a classic experimental design used to test the teratogenic potential of various substances. It is a simple, inexpensive, and quick assay that provides valuable information about the potential risks to developing embryos. Its simplicity, affordability, quick results, sensitivity, and reproducibility are key features. The assay can detect even small doses of teratogens, and it can be used in different laboratories to obtain consistent results. Its reliability and reproducibility make it a valuable tool for assessing the teratogenic potential of various substances. Duck eggs are recruited into the study because a particular defining feature characterizes them, and the researchers hypothesized that exposure to treatment will change that feature.

Ethics in the conduct of this research were strongly considered for the academic integrity of this study. Ethical research practices in educational institutions are strongly followed since it is always the goal of educational research to contribute to the general welfare of the academic community and to generally create measurable information or data that will eventually add to the increase of human knowledge (Ederio, 2023) such as the essence depicted by this study.

RESULTS AND DISCUSSION

A. Phytochemical Components

Table 1. ***Phytochemical components of blue ternate (Clitoria ternatea) leaf extract***

Sample Code	Alkaloids	Anthra-quinones	Cyanogenic-glycosides	Flavonoids	Saponins	Steroids (deoxy-sugars	Tannins
Blue Ternate	+	-	-	+++	+++	+++	+++

Legends:

(-)	**presence is below the detection limit of the method used**
(+)	**presence is only in very small amount**
(++)	**presence is in moderate amount**
(+++)	**presence is very detectable**

The findings show that *Clitoria ternatea* contains flavonoids, saponins, tannins, alkaloids, and steroids in detectable or moderate amounts with no presence of anthraquinones and cyanogenic glycosides. Flavonoids, saponins, steroids (deoxy-sugars), and tannins are present in detectable or moderate quantities, indicating that they may contribute to the potential health benefits of the plant. Alkaloids are present in detectable concentrations and may also be beneficial to health. However, the absence of anthraquinones and cyanogenic glycosides indicates that blue ternatea (*Clitoria ternatea*) is non-toxic and may be safe for human consumption.

Moreover, in the study of Hingtgen (2021), saponins, steroids, tannins, and flavonoids have been shown to possess anti-inflammatory, antimicrobial, antifungal, antiparasitic, insecticidal, and antinutrient properties to protect plants from predators, there is limited evidence suggesting that might affect fetal development when consumed in high concentration.

B. Chorioallantoic Membrane Vascularity Assay

Table 2. ***Number of Primary Blood Vessels of Chorioallantoic Membrane treated with the test extract***

Treatments	Replicates								Total	Mean
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**		
Negative (Distilled Water)	2	3	2	1	1	1	1	3	14	1.75
Positive (Vitamin A)	1	1	1	2	2	0	0	0	7	1.40
100 um extract	1	1	2	1	1	1	1	1	9	1.13
1,000 um extract	2	3	3	3	2	1	1	3	18	2.25
10,000 um extract	1	1	3	2	2	1	2	2	14	1.75

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IJCSRR @ 2024
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Table 2 presents the treatment with the highest mean count, the 1,000 um extract, with a mean value of 2.25. This suggests a potential stimulatory effect on angiogenesis while it is crucial to determine whether this increase in blood vessels is accompanied by any developmental abnormalities or teratogenic effects. The stimulatory effect could be beneficial if it promotes healthy tissue development. In a study by Santos et al. (2018), teratogenicity assessment should include observations of developmental landmarks, organogenesis, and other relevant endpoints to ascertain any potential developmental abnormalities.

On the other hand, the treatment with the lowest mean count is the 100 um extract, with a mean value of 1.13, indicating a relatively weaker effect on blood vessel formation. However, it's essential to emphasize that even treatments with seemingly minimal effects on blood vessel counts which require comprehensive teratogenicity assessment to ascertain their impact on overall embryonic or fetal development.

The data provides valuable insights into the effects of treatments on primary blood vessels count, but assessing teratogenic effects requires a comprehensive approach involving development as evaluations. Teratogenicity studies, often involving controlled animal models and advanced imaging techniques are necessary to draw meaningful conclusions about the potential risks and safety of these treatments during pregnancy (Nazarali et. al.,1998).

Table 3. ***Number of Secondary Blood Vessels of Chlorioallantoic Membrane treated with the test extract***

Treatments	Replicates								Total	Mean
	1	2	3	4	5	6	7	8		
Negative (Distilled Water)	11	14	11	7	3	11	5	13	75	9.38
Positive (Vitamin A)	15	7	7	7	8	0	0	0	44	8.80
100 um extract	2	7	4	2	7	6	7	4	39	4.88
1,000 um extract	5	8	11	10	8	2	2	5	51	6.38
10,000 um extract	6	8	19	12	3	9	7	9	73	9.13

Table 3 presents the treatment with the 10,000 um extract yielded the highest mean value of 9.13 similar to the negative control. This suggests that at this high concentration, the extract's effect on secondary blood vessel development is relatively limited. However, the data exhibits some variability among replicates, implying inconsistencies in the extract's impact. This variability might be attributed to factors such as experimental conditions or individual variation within the CAM.

Conversely, the 100 um extract treatment resulted in the lowest mean value of 4.88 compared to both the negative and positive controls. This substantial decrease in secondary blood vessel development indicates a potential inhibitory effect of the 100 um extract on angiogenesis within the CAM. Studies have shown that altered angiogenesis can result in developmental anomalies, affecting organogenesis and tissue differentiation (Tahergorabi et. al.,2012). This finding raises concerns about possible teratogenic effects at this concentration, as reduced blood vessel formation during embryogenesis could result impaired organ development and function.

The data obtained from the study examining the teratogenic potential of different test extracts on secondary blood vessel development within the choriollantoic membrane (CAM) has provided valuable insights into their potential impacts on embryonic development. Teratogenicity of Natural Compounds have shown that certain natural compounds, including plant extracts, can have teratogenic effects when exposed to developing embryos. Research has demonstrated that certain alkaloids and phytochemicals present in plant extracts can interfere with normal embryonic development (Cevallos et. al.,2022).

Table 4 presents the influence of different extracts on the number of tertiary blood vessels in the CAM. The results showed that the Blue Ternate leaf extract did not have any significant teratogenic effects at concentrations of 100 um and 1,000 um. The highest mean value was observed in the Positive (Vitamin A) treatment indicating that vitamin A is not teratogenic. However, at a concentration of 10,000 um, the extract caused a significant reduction in the number of tertiary blood vessels, suggesting that Blue Ternate leaf extract may have a teratogenic effect at high concentrations. This suggests that the Blue Ternate leaf extract may have the potential to cause birth defects at high concentrations.

Vitamin A has long been recognized for its role in embryonic development and angiogenesis. The study of Arensman et al., (2015) and Saghiri et al., (2005) has shown that Vitamin A can promote endothelial cell proliferation, migration, and tube

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formation, ultimately leading to increased blood vessel growth. The significantly higher mean value observed in the Positive (Vitamin A) treatment supports previous research indicating its angiogenic properties.

Table 4. ***Number of Tertiary Blood Vessels of Chlorioallantoic Membrane treated with the test extract***

Treatments	Replicates								Total	Mean
	1	2	3	4	5	6	7	8		
Negative (Distilled Water)	22	29	16	7	9	12	18	7	120	15.00
Positive (Vitamin A)	52	22	23	26	23	0	0	0	146	29.20
100 um extract	25	20	23	27	21	16	26	28	186	23.25
1,000 um extract	15	13	16	15	11	6	5	7	88	11.00
10,000 um extract	10	17	8	12	11	6	8	8	80	10.00

Table 5 presents the treatment with the 100 um extract demonstrated the highest mean of 29.25, suggesting a significant effect on the total number of blood vessels. The 100 um extract group had a mean of 29.25 blood vessels, which is significantly higher than the negative control group. This suggests that the 100 um extract of Blue Ternate leaf extract may have a beneficial effect on blood vessel development.

The 1,000 um extract group had a mean of 19.63 blood vessels, which is significantly lower than the negative control group. This suggests that the 1,000 um extract of Blue Ternate leaf extract may have a detrimental effect on blood vessel development.

Table 5. ***Total Number of Blood Vessels of Chlorioallantoic Membrane treated with the test extract***

Treatments	Replicates								Total	Mean
	1	2	3	4	5	6	7	8		
Negative (Distilled Water)	35	46	29	15	13	24	24	23	209	26.13
Positive (Vitamin A)	68	30	31	35	33	0	0	0	197	24.63
100 um extract	28	28	29	30	29	23	34	33	234	29.25
1,000 um extract	22	24	30	28	21	9	8	15	157	19.63
10,000 um extract	17	26	30	26	16	16	17	19	167	20.88

The study by Khater et al., (2020) showed that extracts containing bioactive compounds, such as polyphenols and flavonoids, can influence angiogenesis, the process of blood vessel formation. These bioactive compounds possess anti-inflammatory and antioxidant properties, which may promote blood vessel growth and improve vascular health.

In contrast, the treatment with the 1,000 um extract exhibited the lowest mean of 19.63, indicating a relatively weaker influence on blood vessel formation which was supported by the study of Tamanoi et al., (2019) that indicated that the concentration of bioactive compounds in plant extracts can significantly affect their biological activities. It is plausible that the lower concentration of bioactive compounds in the 1,000 um extract may have contributed to its reduced impact on angiogenesis in the Chorioallantoic membrane.

Table 6 shows the percentage vascularity inhibition, which measures the efficiency of varying doses of Blue ternate extract and Vitamin A in preventing blood vessel development.

The treatment with Blue ternate extract at a concentration of 100 um resulted in a vascularity inhibition of -11.96%. This indicates that this concentration of Blue ternate extract stimulated vascularity instead of inhibiting it. This result may suggest a different mechanism of action that promote blood vessel growth at this concentration.

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Table 6. ***Percent Vascularity Inhibition of the Three (3) concentrations of the ethanolic extract of Blue ternate and Vitamin A (positive control)***

Treatment	Total number of Blood Vessels Observed (PBV + SBV + TBV)	Percent Vascularity Inhibition
Negative (Distilled Water)	209	0.00%
Positive (Vitamin A)	197	5.74%
100 um extract	234	-11.96%
1,000 um extract	157	24.88%
10,000 um extract	167	20.10%

On the other hand, a 1,000 um concentration of Blue ternate extract inhibited vascularity by the maximum percentage, 24.88%. It suggests that the 1,000 um extract inhibited vascularity effectively.

The National Cancer Institute (2018) discussed that angiogenesis inhibitors are distinct cancer-fighting compounds because they inhibit the development of blood vessels that support tumor growth rather than tumor cell growth. Anti-angiogenic medicines are treatments that prevent tumors from developing their own blood vessels, which may delay or even reverse the development of the malignancy (Cancer Research UK, 2021). It is also supported by the study of Pathol, A.J (2004) which suggests that the inhibition of VEGF signaling not only prevents tumor angiogenesis but also alters or destroys tumor vessels.

According to the study by Oguis, G. K., et al. (2019), blue ternate substantially inhibits both carrageenin-induced rat paw edema and acetic acid-induced rat vascular permeability. The flavonols and anthocyanins in the plant are responsible for its pharmacological properties.

Table 7. ***Mortality Rate of the embryo after administration of treatment***

Treatment	Number of Embryo/Duck egg Observed	Number of Dead Embryo/Duck egg	Mortality Rate
Negative (Distilled Water)	8	0	0.00%
Positive (Vitamin A)	8	3	37.50%
100 um extract	8	0	0.00%
1,000 um extract	8	0	0.00%
10,000 um extract	8	0	0.00%

Table 7 presents the embryonic mortality rate of embryos after the administration of treatment. It is the proportion of embryos or duck eggs that did not survive after receiving a particular treatment.

The mortality rate of the positive control group administered Vitamin A was 37.50 percent. Three of the eight embryos/duck eggs observed were discovered to be deceased. This indicates that, among all interventions, Vitamin A had the greatest effect on embryo mortality. However, the negative control and all Blue ternate extract concentrations (100 um, 1,000 um, and 10,000 um) exhibited an identical mortality rate of 0%. No deceased embryos or duck eggs were discovered in these groups. This indicates that neither the administration of distilled water (negative control) nor the administration of Blue ternate extracts at varied concentrations resulted in significant embryo mortality.

World Health Organization (2013) recommends a maximum amount of 10,000 IU per day or 25,000 IU per week of vitamin A for women after the first 60 days of pregnancy to be considered safe. Also, the UK National Institute for Health and Clinical Excellence (NICE) recommends that pregnant women don't take vitamin pills with more than 5000 IU (1500 g) of vitamin A. High amounts of vitamin A can cause problems, especially teratogenic effects.

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Different treatments have been studied to determine their impacts on embryonic mortality. The study of Aygun, A. (2016) discovered that injecting embryos with propolis water extract reduced embryonic mortality in Japanese quail.

C. Teratogenicity Assay

Table 8 presents the body weights of duck embryos following 72 hours of incubation under various treatments. The total body mass of all replicates is 13.26 grams, while the average body mass is 1.66 grams. This average value provides an overall representation of the body weight of the embryos in the control group. In the positive control group, duck embryos with varying body weights were treated with Vitamin A wherein some replicates weighed between 1.198 and 1.565 grams, while others weighed zero. The total body mass of all replicates is 6.91 grams, while the average body mass is 1.38 grams. The observed variation in body weights within this group, including some embryos with zero grams, may be attributable to the growth-inhibiting effects of vitamin A.

Table 8. ***Body Weights (in grams) of the Duck Embryos After 72 Hours of Incubation in Different Treatments***

Treatments	Replicates								Total	Mean
	1	2	3	4	5	6	7	8		
Negative (Distilled Water)	1.565	1.594	1.59	1.742	1.697	1.598	1.548	1.892	13.2	1.66
Positive (Vitamin A)	1.198	1.339	1.488	1.565	1.315	0	0	0	6.91	1.38
100 um extract	1.456	1.639	1.452	1.452	1.573	1.459	1.313	1.307	11.65	1.46
1,000 um extract	1.703	1.592	1.366	1.648	1.753	1.379	1.33	1.4	12.17	1.52
10,000 um extract	1.592	1.622	1.702	1.786	1.703	1.758	1.135	1.392	12.69	1.59

A study of Tangara, M. et al., (2010) discover that in-ovo administration of carbohydrates and arginine into the duck amnion improved glycogen stores and perinatal growth, resulting in greater body weights at hatch and 7 days of age. Moreover, a study of Bilalissi, A. et al., (2022) suggest that the embryo weights of eggs stored in a non-ventilation incubator were substantially greater than those of eggs stored in a ventilation incubator. Lastly, a study conducted by Sgavioli S., et al., (2015) discuss that injecting ascorbic acid into incubated eggs exposed to thermal duress improved incubation parameters and infant quality.

Table 9. ***Crown-Rump Length (in mm) of the Duck Embryos after 72 Hours of Incubation in Different Treatments***

Treatment	Replicates								Total	Mean
	1	2	3	4	5	6	7	8		
Negative (Distilled Water)	38.26	37.01	38.34	37.10	39.23	38.40	38.33	36.70	303.37	37.92
Positiv (Vitamin A)	28.49	36.42	36.61	41.25	39.62	0	0	0	182.39	36.48
100 um extract	40.19	41.75	42.50	37.03	38.95	36.90	32.76	31.86	301.94	37.74
1,000 um extract	33.05	36.30	32.79	39.24	38.21	37.00	35.67	37.92	290.18	36.27
10,000 um extract	37.76	38.63	39.11	39.94	40.80	40.74	36.13	40.45	313.56	39.20

Table 9 presents the Crown-Rump Length (CRL) of duck embryos after 72 hours of incubation under various conditions. The CRL values of duck embryos in the group treated with a 10,000 um extract of Blue ternate got the highest value ranging from 36.13 to 40.80 mm. The mean CRL value is 39.20 mm, with a total value of 313.56 mm for all replicates combined.

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At the same time, the lowest CRL values of duck embryos are in the group treated with a 1,000 um extract of Blue ternate ranging from 32.79 to 39.24 mm. The mean CRL value is 36.27 mm, and the total CRL value of all replicates is 290.18 mm.

The crown-rump length is used to measure the length of human embryos and fetuses. It is measured from the cranium (the top of the skull) to the rear (the bottom of the pelvis). A study conducted by Kang, J.Y., et al., (2013) examines the relationship between crown-rump length measured before the 10th week of gestation and birth weight which suggests that the CRL measured in the early first trimester of pregnancy is closely correlated with birth weight, and the accuracy of birth weight prediction is highest on day 67 (9+3 week) of pregnancy.

Table 10 presents the Body Mass Index(BMI) of Duck Embryos after 72 hours of incubation under different treatments. The treatment with the greatest mean value gave the duck embryos their highest average body mass index (BMI). The "1,000 um extract" treatment in this instance had the highest mean, with a mean value of 1.17 mg/mm2. On the other hand, the treatment with the lowest mean value produced duck embryos with the lowest BMI on average. The "Positive (Vitamin A)" treatment has the lowest mean, according to the available data, with a mean value of 1.07 mg/mm2.

Table 10. ***Body Mass Index (in mg/mm2) of the Experimental Duck Embryos after 72 Hours of Incubation in Different Treatments***

Treatments	Replicates								Total	Mean
	1	2	3	4	5	6	7	8		
Negative (Distilled Water)	1.07	1.16	1.08	1.27	1.10	1.08	1.08	1.40	9.25	1.16
Positive (Vitamin A)	1.48	1.01	1.11	0.92	0.84	0	0	0	5.35	1.07
100 um extract	0.90	0.94	0.80	1.06	1.04	1.07	1.22	1.29	8.32	1.04
1,000 um extract	1.56	1.21	1.27	1.07	1.20	1.01	1.05	0.97	9.33	1.17
10,000 um extract	1.12	1.09	1.11	1.12	1.02	1.06	0.87	0.85	8.24	1.03

In a study by Brunstroem., et al (2016) when the duck embryo livers were exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in vitro, EROD was induced by very low concentrations. Moreover, a study by Genet., et al (2018). The effect of BMI on the outcome of in vitro fertilization (IVF) remains unclear. Although some authors have reported no negative effects of obesity on IVF outcomes, others have directly linked overweight and obesity to adverse outcomes. This includes the need for an increased dose of gonadotropins, a reduced number of oocytes collected, a high cancelation rate, and reduced pregnancy and live birth rates.

Table 12 presents the analysis of the head-beak length-to-body length ratio of the duck embryos after 72 hours of incubation under different treatments. The treatment with the highest mean value was the "10,000 um extract" with a mean value of 0.39 mm. In contrast, the treatment with the lowest mean value was the "1,000 um extract" with a mean value of 0.31 mm.

Table 11. ***Head-Beak Length (in mm) to Body Length (in mm) Ratio of the Duck Embryos after 72 Hours of Incubation in Different Treatments***

Treatments	Replicates								Total	Mean
	1	2	3	4	5	6	7	8		
Negative (Distilled Water)	1.07	1.16	1.08	1.27	1.10	1.08	1.08	1.40	9.25	1.16
Positive (Vitamin A)	1.48	1.01	1.11	0.92	0.84	0	0	0	5.35	1.07
100 um extract	0.90	0.94	0.80	1.06	1.04	1.07	1.22	1.29	8.32	1.04
1,000 um extract	1.56	1.21	1.27	1.07	1.20	1.01	1.05	0.97	9.33	1.17
10,000 um extract	1.12	1.09	1.11	1.12	1.02	1.06	0.87	0.85	8.24	1.03

The study conducted by Pastor and Almadin et al. (2017) shows the effects of different concentrations of an extract on duck embryos were investigated. The extract was tested at concentrations of 0.12 g/mL, 0.24 g/mL, 0.35 g/mL, and 0.47 g/mL, corresponding to 25%, 50%, 75%, and 100% concentrations, respectively. The 75% and 100% concentrations also exhibited mostly

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zero development of the embryos, leaving low morphometric results which are also significantly different from the control group ($p<0.05$). In comparison between treatments, morphometric analysis under the head-beak length measurement also showed significant differences in between the 25% and 100% concentration.

These findings are particular to the provided data and may call for additional study and context to reach reliable conclusions. Additionally, a more thorough analysis of the findings would result from knowing the significance of the head-beak length-to-body length ratio used in the study.

Table 12. ***Forelimb Length (in mm) to Body Length (in mm) Ratio of the Duck Embryos after 72 Hours of Incubation in Different Treatments***

Treatment	Replicates								Total	Mean
	1	2	3	4	5	6	7	8		
Negative (Distilled Water)	0.23	0.25	0.25	0.25	0.23	0.25	0.26	0.17	1.92	0.24
Positive (Vitamin A)	0.15	0.26	0.27	0.40	0.37	0	0	0	1.48	0.29
100 um extract	0.41	0.37	0.37	0.24	0.21	0.30	0.19	0.17	2.30	0.28
1,000 um extract	0.49	0.17	0.28	0.24	0.25	0.25	0.26	0.23	2.19	0.27
10,000 um extract	0.29	0.26	0.29	0.26	0.21	0.40	0.33	0.37	2.45	0.30

Table 12 illustrates the forelimb length to body length ratio of the duck embryos after 72 hours of incubation using the information in the table shown and the various treatments. The treatments listed in the table are Distilled Water (Negative), Vitamin A (Positive), 100 um, 1,000 um, and 10,000 um extracts. Replicates, total values, and mean values are also shown in the table for each treatment. The forelimb length to body length ratio of the duck embryos is often the highest for the treatment with the highest mean value. According to the information presented, the therapy of 10,000 um extract has the highest mean, with a mean value of 0.30 mm.

On the other hand, the forelimb length to body length ratio of the duck embryos is, on average, the lowest in the treatment with the lowest mean value. The Positive (Vitamin A) treatment has the lowest mean value among the treatments based on the provided data, with a mean value of 0.29 mm.

A study by Gouda., et al (2022) highlighted that *all in-ovo* injected groups with vitamin A, L-carnitine, and folic acid increased the embryo weight, residual yolk weight, heart weight, hatchability percentage, and embryo length at the 25th day of incubation. Conclusively, the *in ovo* feeding of the present micronutrients showed positive impacts on embryonic development, and hatchling health status of ducklings.

Table 13 illustrates the hind leg length to body length ratio of the duck embryos after 72 hours of incubation using the information based on the table shown and the various treatments. The table lists the following treatments: 100 um extract, 1,000 um extract, 10,000 um extract, Positive (Vitamin A), Negative (Distilled Water), and Positive (Ultrasound). Replicates, total values, and mean values are also shown in the table for each treatment. The majority of the duck embryos in the treatment with the highest mean value have the greatest hind limb length to body length ratio. The 10,000 um extract therapy has the highest mean according to the information presented, with a mean value of 0.34 mm. On the other hand, the hind leg length to body length ratio of the duck embryos is, on average, the lowest in the treatment with the lowest mean value. The 1,000 um extract treatment has the lowest mean value out of the given treatments, with a mean value of 0.29 mm.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 07 Issue 05 May 2024
DOI: 10.47191/ijcsrr/V7-i5-69, Impact Factor: 7.943
IJCSRR @ 2024
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Table 13. ***Hind Limb Length (in mm) to Body Length (in mm) Ratio of the Duck Embryos after 72 Hours of Incubation in Different Treatments***

Treatment	Replicates								Total	Mean
	1	2	3	4	5	6	7	8		
Negative (Distilled Water)	0.27	0.25	0.26	0.27	0.31	0.30	0.30	0.17	2.17	0.27
Positive (Vitamin A)	0.24	0.28	0.31	0.35	0.37	0	0	0	1.58	0.31
100 um extract	0.44	0.43	0.42	0.34	0.24	0.32	0.19	0.16	2.57	0.32
1,000 um extract	0.18	0.22	0.37	0.29	0.30	0.36	0.30	0.34	2.39	0.29
10,000 um extract	0.32	0.32	0.29	0.32	0.33	0.39	0.36	0.38	2.75	0.34

The study by Yildirim., et al (2018) presented that the key features associated with slower embryonic development in ducks have not been adequately described. This study aimed to characterize the pattern and the speed of early embryogenesis in Brown Tsaiya Ducks (BTD) compared with those in Taiwan Country Chicken (TCC) by using growth parameters including embryonic crown-tail length (ECTL), primitive streak formation, somitogenesis, and other development-related parameters, during the first 72 h of incubation.

Moreover, The results showed that from 84 normally developing chick embryos, 5 were randomly chosen each day from incubation days 5 to 18 and scanned using 3.0 Tesla magnetic resonance imaging (Zhou., et. al., 2017). A dual-cooling technique is used before and during imaging. Eggs were cracked for making histological specimens after imaging, and 3 eggs were serially imaged from days 5 to 18. It shows that skeletal muscle fibers can be tracked in the hind limb in DTI beginning with incubation day 8. The data shows a good positive correlation between quantitative DTI and histologic parameters (FA vs Fiber_Area%: r= 0.943, $p<0.0001$; Fiber_length vs Limb_length: r=0.974, $p<0.0001$). Also, the result of tracked fibers in DTI during incubation corresponds to the development of chick embryonic skeletal muscle as reported in the literature.

Table 14 presents the significant differences in results Using Analysis of Variance ANOVA among treatments of the measured indicators. The result shows no significant differences among treatments on the Primary Blood Vessels, Secondary Blood Vessels, Body Weight, Body Mass Index, Crown-Rump Length, Head-Beak Length to Body Length Ratio, Fore Limb to Body Length Ratio, and Hind Limb Length to Body Length Ratio.

D. Significant Difference Between the Control and Experimental Set-Ups

Table 14. ***Presentation of Significant Differences Results Using Analysis of Variance ANOVA among treatments***

	Test Statistic (F)	p-value	Decision	Interpretation
Ho: There is no significant difference on the Primary Blood Vessels among treatments	2.651	.050	Do not Reject Ho	Not Significant
Ho: There is no significant difference on the Secondary Blood Vessels among treatments	2.276	0.083	Do not Reject Ho	Not Significant
Ho: There is no significant difference on the Tertiary Blood Vessels among treatments	9.936	0.000	Reject Ho	Significant

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 07 Issue 05 May 2024
DOI: 10.47191/ijcsrr/V7-i5-69, Impact Factor: 7.943
IJCSRR @ 2024
www.ijcsrr.org

Ho: There is no significant difference on the Body Weights among treatments	3.080	.030	Do not Reject Ho	Not Significant
Ho: There is no significant difference on the Crown-Rump Length among treatments	1.254	.308	Do not Reject Ho	Not Significant
Ho: There is no significant difference on the Body Mass Index among treatments	1.206	.327	Do not Reject Ho	Not Significant
Ho: There is no significant difference on the Head- Beak Length to Body Length Ratio among treatments	2.075	.107	Do not Reject Ho	Not Significant
Ho: There is no significant difference on the Fore Limb to Body Length Ratio among treatments	.881	.486	Do not Reject Ho	Not Significant
Ho: There is no significant difference on the Hind Limb Length to Body Length Ratio among treatments	1.311	.287	Do not Reject Ho	Not Significant

This implies that the Leaf extract of Blue Ternate did not significantly affect the earlier-mentioned indicators. However, there is insufficient evidence to show that the test extracts have a significant effect on Tertiary Blood Vessels.

Several studies have investigated the development and characteristics of blood vessels in various contexts. For instance, Naito et al., (2020) conducted a study on blood vessel formation during embryonic development, examining the role of growth factors and signaling pathways. Their findings demonstrated the importance of precise regulation in primary and secondary blood vessel formation. These results align with the present study's finding of no significant differences in primary and secondary blood vessels among the treatments, suggesting that the treatments did not significantly impact the development or morphology of these blood vessel types.

In contrast to the study of Vergara et al., (2021) they examined the effects of different treatments on the microvasculature and angiogenesis. They observed that specific treatments led to significant changes in tertiary blood vessels, indicating treatment-induced alterations in the exemplary vascular network. This aligns with the present study's results, which revealed a significant

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ISSN: 2581-8341
Volume 07 Issue 05 May 2024
DOI: 10.47191/ijcsrr/V7-i5-69, Impact Factor: 7.943
IJCSRR @ 2024
www.ijcsrr.org

difference in tertiary blood vessels among the treatments. These findings suggest that the treatments in the current study exerted distinct effects on the formation or characteristics of tertiary blood vessels, potentially influencing angiogenesis processes.

While no specific studies were mentioned for body mass index, body weight, crown-rump length, head-beak length-to-body length ratio, fore limb-to-body length ratio, and hind limb length-to-body length ratio in the provided data, it is essential to consider that these parameters are commonly investigated in fields such as developmental biology, zoology, or anthropometry. A comprehensive review of the literature within these domains may provide insights into factors affecting these morphological measurements.

CONCLUSION AND RECOMMENDATIONS

The following conclusions have been drawn based on the study's findings:

This study indicates that *Clitoria ternatea* is a plant rich in phytochemicals like alkaloids, tannins, and steroids, showcasing antioxidant, anti-inflammatory, antibacterial, and potential chemotherapeutic properties. The absence of anthraquinones and cyanogenic glycosides suggests a low consumption risk. Alkaloids exhibit antimicrobial, antidiabetic, and anticancer effects, while tannins show antioxidant, anti-inflammatory, and antimicrobial activities. Steroids display antioxidant, anti-inflammatory, and anticancer properties. Despite some exploration of *Clitoria ternatea's* biological activities, more research is essential to comprehensively understand their mechanisms and therapeutic applications. *Clitoria ternatea* shows promise as a source of different phytochemicals that could be used in chemotherapeutics. However, more research is needed to fully understand how they work and how they can be used in therapy.

Moreover, the study's results show that the treatment may have caused teratogenicity in tertiary blood vessels, as shown by the fact that there were significant differences and the null hypothesis was rejected using ANOVA statistics. This is concerning, as damage to these vessels can lead to developmental abnormalities in the growing fetus. Tertiary blood vessels play a crucial role in supplying blood to developing organs, and any disruption could have serious consequences. Additionally, the treatment's angiosuppressive effects on these vessels are significant, as angiogenesis is essential for fetal growth. Further investigation is necessary to understand the specific impacts on tertiary blood vessels, unveil mechanisms of teratogenicity and angiosuppression, and develop strategies to mitigate potential harm to fetal development.

Lastly, the study also compared treatment effects of the body length where it shows no significant difference between the treatments in terms of body mass index, body weight, crown-rump length, head-beak length to body length ratio, fore limb to body length ratio, or hind limb length to body length ratio.

Based on the research findings of "Teratogenic Risk Potentiality Of Blue Ternate (*Clitoria Ternatea*) Leaf Extract using Chorioallantoic Membrane (Cam) Assay: Phase One", the following recommendations can be made:

Medical Practitioners

A comprehensive program aimed at disseminating information about the health benefits of Blue Ternate leaf for embryonic development should include educational workshops, informative materials, digital outreach, collaboration with healthcare providers, community engagement programs, research and documentation, partnerships with women's health organizations, and continuous monitoring and feedback. This approach will raise awareness, educate, and promote the integration of Blue Ternate leaf into dietary and health practices among pregnant women, promoting positive dietary choices and embryonic development. In addition, a comprehensive research study should be conducted to explore the impact of refining and storage on the teratogenic potential of Blue Ternate. This includes a literature review, experimental design, sample collection and preparation, chemical analysis, biological assays, histopathological examination, statistical analysis, quality control measures, ethical considerations, and interpretation and reporting. This investigation can provide valuable insights into the safety profile and potential guidelines for its use in various applications.

The Public

Consult a healthcare professional before using Blue Ternate for medical treatment. They can evaluate individual health profiles, medications, and potential interactions. Transparent communication of medical history helps physicians make informed decisions. Consultation is crucial for personalized advice and a comprehensive approach to overall well-being.

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ISSN: 2581-8341
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DOI: 10.47191/ijcsrr/V7-i5-69, Impact Factor: 7.943
IJCSRR @ 2024
www.ijcsrr.org

Future Researchers

The MMT assay is used to study the anti-cancer potential of Blue Ternate leaf extract. It measures mitochondrial enzyme reduction, providing insights into its mechanisms of action. This research could lead to potential therapeutic applications and further exploration. And a study evaluating the hypoglycemic effect of Blue Ternate leaf extract on white mice is crucial for understanding its potential benefits in managing blood glucose levels. The study will use standardized administration protocols, regular blood glucose monitoring, and a long-term study duration. The findings will contribute to the growing body of knowledge on Blue Ternate's medicinal properties and may have implications for future therapeutic developments related to diabetes and glucose regulation. Also, research on Blue Ternate's teratogenicity in its root, stem, sap, and bud parts is crucial for understanding its safety profile. In-depth analyses using techniques like chromatography, mass spectrometry, and spectroscopy can identify active ingredients and their potential teratogenic effects. This approach can help determine safe and unsafe components, guiding informed consumption decisions and potential applications.

The research should also consider incorporating additional indicators beyond vascular density, embryo weight, and morphometric indices to better understand the potential teratogenic effects of Blue Ternate leaf extract on embryonic development. This will enhance the robustness and depth of the study, providing a more nuanced understanding of the potential effects on embryonic development and the safety profile of Blue Ternate leaf extract. And, researchers should explore the effects of Blue Ternate on various animal models to better understand its potential impact on physiological processes. Regular administration of Blue Ternate extracts has been linked to increased levels of acetylcholine, a crucial neurotransmitter for brain health. This research could lead to more targeted applications in neuroscience, neuropharmacology, and cognitive health.

Moreover, a comparative analysis of Blue Ternate flowers and leaves needs to be conducted to understand their teratogenicity and therapeutic potential. The study will involve a structured experimental design, teratogenicity assessments, biochemical analysis, histopathological studies, pharmacological studies, statistical analyses, and ethical considerations. The findings will inform future research, guide recommendations, and provide a nuanced understanding of the plant's safety and therapeutic potential.

Lastly is to conduct a descriptive survey on pregnant women who have consumed Blue Ternate, aiming to establish correlations with the study's findings. The survey will collect data on maternal morbidity, mortality, and health outcomes, and will be analyzed using statistical methods. The results will help guide recommendations for the plant's use in prenatal care and provide a comprehensive understanding of its potential risks.

ACKNOWLEDGEMENT

This journey of experimentation, fraught with late nights, data dances, and the thrill of discovery, culminates not only in results but in a symphony of gratitude. From the spark of an idea to the final analysis of data, has been a tapestry woven with countless threads of support, each deserving its own note of appreciation.

First and foremost, we express our gratitude to God for the divine hand that grants us the opportunity to embark on this journey. Our deepest appreciation to the Panelists for their insightful questions and feedback, which challenged us to refine their arguments and see their work through a clear lens. The Statistician, Ms. Eva A. Rellon, brought order to the chaos of data by translating the whispers of observations into statistical melodies, revealing patterns and insights invisible to the naked eye. Her skill and patience made sense of the unknown, giving voice to the data and lending credence to our findings.

We would be remiss not to express our heartfelt gratitude to our Research Adviser, Ms. Carmel Grace B. Guazon, RN, for her mentorship, guidance, and encouragement. Dr. Nikko T. Ederio, the dedicated Research Instructor, who was the first line of defense against technical hurdles and answered our endless questions with unflagging enthusiasm. Both of their expertise was invaluable, and their spirit contagious, making even the most challenging tasks feel like a shared adventure.

We express their deep bow to the CSU Biology and Chemistry Laboratory for providing the equipment and resources that brought our research to life. Their dedication to scientific inquiry created an environment of collaboration and discovery, where ideas could flourish and take shape.

Finally, we turn to our family and loved ones for their unwavering support and belief in us. Their love was the fuel that kept us going, reminding us that this research was not just about scientific discovery but about contributing something meaningful to the world we share.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 07 Issue 05 May 2024
DOI: 10.47191/ijcsrr/V7-i5-69, Impact Factor: 7.943
IJCSRR @ 2024
www.ijcsrr.org

This research is a testament to the power of collaboration and support. You are the notes that make up this melody, the voices that weave together the tapestry of our journey. Thank you for being a part of it.

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International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 07 Issue 05 May 2024
DOI: 10.47191/ijcsrr/V7-i5-69, Impact Factor: 7.943
IJCSRR @ 2024
www.ijcsrr.org

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Cite this Article: Rheajean M. Jawel, Sylphjenn C. Orquillo, Xycris Ezenette M. Barcos, Carmel Grace B. Guazon, Arjune A. Lumayno, Nikko T. Ederio (2024). Teratogenic Risk Potentiality of Blue Ternate (Clitoria Ternatea) Leaf Extract Using Chorioallantoic Membrane (CAM) Assay: Phase One. International Journal of Current Science Research and Review, 7(5), 3095-3111