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Determination of Antiteratogenic Potential of *Garcinia mangostana* Linn. (Mangosteen) Fruit Hull Extract on Gross Morphology of Developing *Anas platyrynchos* (Duck) Embryo

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Abstract:

Two crude extract of Mangosteen (Garcinia mangostana) fruit hull, using 95% and 50% ETOH were evaluated for their antiteratogenic effect against the teratogen retinoic acid. 0.2ml of each 10, 50 and 100 µg/ml dosages of both mangosteen crude extract showed no teratogenic activity on the developing 3-day old duck embryos. On the contrary, both extracts administered with retinoic acid showed antiteratogenic properties by minimizing the malformations on the duck embryo's head width, eye diameter, culmen length, culmen depth, radius and ulna length, wing length, body lenth, total body length, shank length and foot length. Statistics shows that the potential antiteratogenic activity seems more effective in higher amounts (50 µg/ml and 100µg/ml) of both extracts, than in the lower concentration (10 µg/ml). Mangosteen crude extract, extracted using 95% ETOH has higher yield of altering malformations caused by retinoic acid than mangosteen crude extract, extracted using 50% ETOH.

Mechanism for the specific antiteratogenic action of the two mangosteen crude extracts remains to be analyzed.

Key words: antiteratogenic, teratogenic, *Garcinia mangostana*, *Anas platyrynchos* biological assay

I. INTRODUCTION

Different kinds and varieties of synthetic drugs are emerging in the market without proper labeled and precautions of usage. Drugs like retinoic acids, an active component in cosmetics and acne treating problems, has an oral form called Accutane. Retinoic acid was proved to be teratogenic when huge amount was taken that can cause abnormalities in embryo's morphology (McCaffery 2006).

According to the 2003 Philippine Health Statistics of the Department of Health, congenital heart malformations and other types of malformations rank fourth, seventh respectively with the mortality rate of 4.9, and 2.0 correspondingly for 1,000 live births.

Pharmaceutical and cosmetic companies should provide proper precautionary labels regarding to their product's side effects, so that proper usage of it will catch consumer's attention. Moreover, further researches are in need to identify genetic variants that predict susceptibility to teratogenic effects (Polifka and Fiedman 2002) and also studies on antiteratogenic substances must be fully explored so that harmful exposures can be prevented.

Scientist at the present time are looking forward to medicinal plants in treating and preventing congenital abnormalities and diseases. The use of natural products as a source of medicines has gained wide acceptance in recent years. These products, especially those isolated from plants, provide novel and clinically active compounds useful in the treatment

and prevention of diseases. One of the well documented plants exhibiting various medicinal actions is *Garcinia mangostana* more commonly known as mangosteen (Asai *et al.*1995).

Garcinia mangostana, a tropical fruit native to Southeast Asia, is known to its medicinal properties. Filipinos employ decoction of the fruit hull, leaves and bark, of this plant as a febrifuge and to treat various ailments such as dysentery, diarrhea, haemorrhoids, food allergies, arthritis, wounds, skin infections, tuberculosis, inflammation, mouth aphthae, fever, amoebic dysentery, eczema, acne, thrush, abdominal pain, suppuration, leucorrhoea, cholera, convulsants, ulcers, micosis, infections of the genitor-urinary tracts, gonorrhoea, cystitis, and urethra suppuration (Chaverri *et al*. 2008 and Quisumbing 1978).

Several studies have shown that extracts obtained from mangosteen fruit hull have remarkable biological and pharmacological properties such as antibacterial (Sakagami et al. 2005), anti-acne inducing bacteria activity (Pothitirat et al. 2009) anti-inflamatory (Chen et al. 2007), antihistamine (Chairungsrilerd et al. 1996), antimutagenic and anticarcinogenic (Chaverri et al. 2005).

Research also shows that 95% and 50% ethanol extracts from the fruit hull of G. mangostana contains antioxidative and neuroprotective property (Weecharangsan et al. 2005). As an addition, mangosteen fruit hull extracts can also inhibit histamine release, prostaglandin E2 synthesis and HIV-1 protease (Chen et al. 2007).

Although several properties of G. mangostana have been studied, there is no study or research available claiming that it is either a teratogen or it has an antiteratogenic property. Thus this study aims to test if Garcinia mangostana Linn. has an antiteratogenic potential that can alter or prevent teratogenic drugs, specifically the retinoic acid.

This study aimed to (1.) identify the morphological abnormalities that can be prevented by the mangosteen's fruit hull extract in the gross morphology (head, eyes, beak, neck, body, wings and legs) of Anas platyrynchus (duck) embryo by means of morphometric analysis. (2.) know the dosage of Mangosteen's fruit hull extract that can alter the effects of 1 µg/µL retinoic acid solution treated on three-day old duck embryo. (3.) Compare the efficacy of the two extract, the mangosteen crude extract, extracted using 95% ETOH and mangosteen crude extract, extracted using 50% ETOH, in altering the effects of 1 µg/µL retinoic acid solution on three-day old duck embryo.

II. METHODOLOGY

2.1 Test Organism

75 newly laid duck eggs were randomly selected from Pateros for this experiment. Chosen eggs were cleansed using 50% ETOH before incubating for 72 hours.

2.2 Plant Extraction

Garcinia mangostana (Mangosteen) fruits bought from the Nepa-Q Mart Public Market were used. Representative fruit samples were submitted to the Botany Section of the National Museum for verification purposes.

Fruits were washed and were chopped into small pieces before they were subjected to air drying for five days (Kosem et al. 2007). Dried hulls were homogenized using a manual oil expellr. The powdered fruit hull were divided into two, the first one was immersed in 95% ETOH, while the second half was soaked in 50% ETOH. Both macerated solution were stayed for two weeks before they were subjected for filtering using Whatman no. 1 filter paper. The filtrates were concentrated under the reduced pressured at 60 – 65^{0}C using rotary vacuum

evaporator at the Chemicals and Minerals Division Building of the Department of Science and Technology.

Afterwards, the two crude extracts were individually evaporated on a boiling water bath, with temperature, not greater than 100^0C until a constant weight was obtained (Pothitirat *et al.* 2009). The resulted dried crude extracts were subjected to pounding using mortar and pestle and were kept until used.

2.3 Experimental Design

3 –day old duck eggs were divided into four groups. Each set-ups contain five replicates.

Group I = the negative control group, which was composed of two set-ups:

NT = no treatment
PBS = contain 0.2ml of Phosphate-Buffered Saline solution

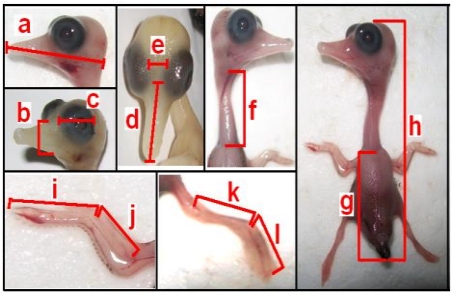
Group II = the Positive control group, which contain 1µl of Retinoic Acid (RA)

Group III = the group treated with mangosteen crude extracts only. It is composed of six subgroups:

A1= 0.2ml of 10 µg/ml mangosteen crude extract, using 95%ETOH
A2 =0.2ml of 50 µg/ml mangosteen crude extract, using 95%ETOH
A3 =0.2ml of 100 µg/ml mangosteen crude extract, using 95%ETOH

B1= 0.2ml of 10 µg/ml mangosteen crude extract, using 50%ETOH
B2 =0.2ml of 50 µg/ml mangosteen crude extract, using 50%ETOH
B3 =0.2ml of 100 µg/ml mangosteen crude extract, using 50%ETOH

Group IV = the group treated with mixed retinoic acid and mangosteen crude extracts. . It is composed of six subgroups:



AR1=1µl RA mixed with 0.2ml of 10 µg/ml mangosteen crude extract, using 95%ETOH
AR2 =1µl RA mixed with 0.2ml of 50 µg/ml mangosteen crude extract, using 95%ETOH
AR3 =1µl RA mixed with 0.2ml of 100 µg/ml mangosteen crude extract, using 95%ETOH

BR1=1µl RA mixed with 0.2ml of 10 µg/ml mangosteen crude extract, using 50%ETOH
BR2 =1µl RA mixed with 0.2ml of 50 µg/ml mangosteen crude extract, using 50%ETOH
BR3 =1µl RA mixed with 0.2ml of 100 µg/ml mangosteen crude extract, using 50%ETOH

2.4 Inoculation Procedure

Aseptically, a hole was made within the area of the air by boring slowly a sterile blood lancet. Various amounts of the test solution was aseptically introduce to each corresponding eggs by using 1cc/ml Luer SlipTip syringes. After solutions were administered, a strip of clay was used to cover the hole where the injection was made.

The eggs were incubated at 37^0C until the duck embryo attained two weeks of incubation. Test embryo will be evaluated and photo documented in terms of their gross morphology development after harvesting. Preservation of the duck embryos were done using 10% formalin.

2.5 Morphometric Analysis of the Duck Embryo

Analysis is composed of twelve morphological characters were measured: head length; eye diameter; spaces between the eyes; culmen length; culmen depth; neck length; radius and ulna; the carpus, metacarpus and digits; body length; total body length; shank (tarsus) length; and foot (phalanges) length (San Diego 2008; and Martines and Cabullo 2006).

2.6 Statistical Analysis

Data collected were statistically analyzed using One way Anova of the SPSS Software Package ver. 16.0 to analyzed the efficacy of each plant dosage and in comparing the effectiveness of the two mangosteen crude extracts, using 95% ETOH and 50% ETOH. Moreover, mean separation was done using Tukey HSD test.

III. RESULTS

This study was set at the 99% confidence level (p=0.01). Computations using One- way ANOVA had determined the differences between the means of the duck embryos morphometry. Results showed that almost of the measured morphological traits were significant at 99% confidence level (p=0.01), except from the space between the eyes (p=0.046) when compared in each treated and control group.

The following morphological trait showed significant values when compared to the RA group:

Head width

Almost all of the negative control and treated groups were significant (p=0.000) on the measured head width of the duck embryo, when compared to the RA group.

Other groups that contain significant values were the groups A1 (p=0.000), A2 (p=0.001) and A3 (p=0.000) when compared to the groups BR1, BR2 and BR3 respectively.

Eye Diameter

In comparing the duck embryos left side eye diameter, results showed that most of the treatments and negative control groups were significant (p=0.000 and p=0.001) in comparison to the RA group. However, the groups AR1 (p=0.020), BR1 (p=0.181), and BR2 (p=0.054) were not significant to the RA group.

Table 1. Shows the Average Morphological Traits Measured from the Duck Embryos in Different Treatments

Duck Embryo Body Parts	Treatments								
	NT	PBS	RA	A1	A2	A3	B1	B2	B3
*Head Width	22.30 ± 0.18	20.92 ± 0.38	11.43 ± 2.11	23.50 ± 0.48	23.12 ± 0.02	23.49 ± 0.21	21.28 ± 0.22	21.70 ± 0.27	21.70 ± 0.28
*Eye Diameter (left side)	8.29 ± 0.18	8.06 ± 0.03	5.54 ± 0.86	8.45 ± 0.26	8.10 ± 0.03	8.46 ± 0.25	7.87 ± 0.18	7.86 ± 0.17	7.86 ± 0.18
*Eye Diameter (right side)	8.30 ± 0.18	8.09 ± 0.03	5.68 ± 1.07	8.44 ± 0.23	8.08 ± 0.04	8.44 ± 0.25	7.89 ± 0.18	7.84 ± 0.17	7.85 ± 0.17
*Space Between the Eyes	2.24 ± 0.20	2.45 ± 0.24	3.46 ± 0.49	2.05 ± 0.29	2.24 ± 0.19	2.26 ± 0.36	2.31 ± 0.18	2.26 ± 0.20	3.04 ± 0.01
*Culmen Length	8.09 ± 0.03	7.66 ± 0.24	4.23 ± 1.23	7.88 ± 0.36	8.05 ± 0.31	8.12 ± 0.03	7.87 ± 0.21	7.64 ± 0.50	7.49 ± 0.23
*Culmen Depth	5.86 ± 0.20	5.68 ± 0.23	4.67 ± 0.72	5.68 ± 0.23	6.06 ± 0.02	5.89 ± 0.18	5.86 ± 0.18	5.46 ± 0.24	5.66 ± 0.25
*Neck Length	14.86 ± 0.38	13.69 ± 0.41	9.44 ± 1.21	15.87 ± 0.46	13.84 ± 1.99	16.25 ± 0.19	13.63 ± 0.40	13.86 ± 0.49	13.81 ± 0.59
*Radio and Ulna (left side)	6.06 ± 0.01	5.89 ± 0.17	3.24 ± 1.13	6.08 ± 0.01	6.04 ± 0.31	5.87 ± 0.21	6.06 ± 0.02	5.84 ± 0.49	5.69 ± 0.24
*Radio and Ulna (right side)	6.08 ± 0.03	5.67 ± 0.25	3.24 ± 1.13	6.06 ± 0.01	5.86 ± 0.36	5.89 ± 0.21	6.06 ± 0.01	6.06 ± 0.31	5.68 ± 0.23
*Wing Length (left side)	8.29 ± 0.18	7.87 ± 0.20	3.62 ± 1.51	8.44 ± 0.24	7.86 ± 0.21	8.44 ± 0.24	7.07 ± 0.02	7.04 ± 0.44	7.64 ± 0.39
*Wing Length (right side)	8.29 ± 0.19	7.68 ± 0.25	4.04 ± 1.32	8.49 ± 0.24	8.04 ± 0.01	8.48 ± 0.25	7.08 ± 0.02	6.85 ± 0.81	7.26 ± 0.19
*Body Length	23.52 ± 0.21	22.48 ± 0.61	17.87 ± 1.35	25.45 ± 0.67	24.49 ± 0.57	24.67 ± 0.78	21.91 ± 0.61	22.08 ± 0.72	22.14 ± 0.57
*Total Body Length	50.66 ± 0.25	47.46 ± 0.69	37.63 ± 4.66	52.85 ± 1.87	52.63 ± 0.51	53.44 ± 1.35	48.06 ± 0.53	49.02 ± 1.45	46.44 ± 0.52
*Shank Length (left side)	6.08 ± 0.02	5.66 ± 0.23	2.86 ± 0.97	6.07 ± 0.03	6.04 ± 0.01	6.26 ± 0.20	5.26 ± 0.19	5.63 ± 0.51	5.06 ± 0.32
*Shank Length (right side)	6.07 ± 0.03	5.86 ± 0.19	3.65 ± 0.67	6.25 ± 0.20	6.43 ± 0.39	6.22 ± 0.20	5.28 ± 0.19	5.86 ± 0.65	5.06 ± 0.32
*Phalanges Length (left side)	9.26 ± 0.19	8.30 ± 0.18	2.61 ± 1.61	9.07 ± 0.32	8.67 ± 0.38	8.66 ± 0.25	7.68 ± 0.38	8.26 ± 0.19	7.85 ± 0.19
*Phalanges Length (right side)	9.06 ± 0.31	8.27 ± 0.20	3.83 ± 1.57	9.04 ± 0.31	8.47 ± 0.38	8.64 ± 0.25	7.68 ± 0.38	7.86 ± 0.20	8.03 ± 0.32

B1= 0.2ml of 10 µg/mL mangosteen crude extract, using 50%ETOH
B2 =0.2ml of 50 µg/mL mangosteen crude extract, using 50%ETOH
B3 =0.2ml of 100 µg/mL mangosteen crude extract, using 50%ETOH

BR1= 0.2ml of 10 µg/mL mangosteen crude extract, using 50%ETOH
BR2 =0.2ml of 50 µg/mL mangosteen crude extract, using 50%ETOH
BR3 =0.2ml of 100 µg/mL mangosteen crude extract, using 50%ETOH

Duck Embryo Body Parts	Control Groups			Treatments					
	NT	PBS	RA	AR1	AR2	AR3	BR1	BR2	BR3
*Head Width	22.30 ± 0.18	20.92 ± 0.38	11.43 ± 2.11	20.68 ± 0.90	23.48 ± 1.16	22.70 ± 0.67	17.62 ± 1.25	18.82 ± 0.98	20.05 ± 0.33
*Eye Diameter (left side)	8.29 ± 0.18	8.06 ± 0.03	5.54 ± 0.86	7.46 ± 0.38	8.05 ± 0.43	8.10 ± 0.48	7.03 ± 0.31	7.28 ± 0.37	7.85 ± 0.21
*Eye Diameter (right side)	8.30 ± 0.18	8.09 ± 0.03	5.68 ± 1.07	7.62 ± 0.24	7.83 ± 0.81	8.23 ± 0.20	7.21 ± 0.38	7.29 ± 0.37	7.84 ± 0.20
*Space Between the Eyes	2.24 ± 0.20	2.45 ± 0.24	3.46 ± 0.49	2.28 ± 0.20	2.46 ± 0.23	2.26 ± 0.22	3.02 ± 0.32	2.46 ± 0.49	2.67 ± 0.23
*Culmen Length	8.09 ± 0.03	7.66 ± 0.24	4.23 ± 1.23	6.03 ± 0.64	7.66 ± 0.24	7.84 ± 0.37	5.63 ± 0.60	5.86 ± 0.75	6.44 ± 0.40
*Culmen Depth	5.86 ± 0.20	5.68 ± 0.23	4.67 ± 0.72	5.63 ± 0.24	5.85 ± 0.36	5.05 ± 0.01	3.82 ± 0.60	5.06 ± 0.44	5.25 ± 0.38
*Neck Length	14.86 ± 0.38	13.69 ± 0.41	9.44 ± 1.21	14.25 ± 1.91	14.07 ± 1.13	14.42 ± 0.92	12.62 ± 1.08	11.84 ± 1.13	12.24 ± 0.49
*Radio and Ulna (left side)	6.06 ± 0.01	5.89 ± 0.17	3.24 ± 1.13	5.25 ± 0.48	5.86 ± 0.36	6.46 ± 0.22	4.03 ± 1.06	4.84 ± 0.39	5.46 ± 0.42
*Radio and Ulna (right side)	6.08 ± 0.03	5.67 ± 0.25	3.24 ± 1.13	4.86 ± 0.22	5.84 ± 0.37	6.45 ± 0.23	4.02 ± 1.06	4.84 ± 0.38	5.45 ± 0.40
*Wing Length (left side)	8.29 ± 0.18	7.87 ± 0.20	3.62 ± 1.51	5.85 ± 1.47	7.64 ± 0.24	8.23 ± 0.38	3.82 ± 1.57	6.04 ± 0.32	7.06 ± 0.31
*Wing Length (right side)	8.29 ± 0.19	7.68 ± 0.25	4.04 ± 1.32	5.86 ± 0.82	7.43 ± 0.24	8.10 ± 0.31	5.64 ± 1.44	5.24 ± 0.38	7.06 ± 0.02
*Body Length	23.52 ± 0.21	22.48 ± 0.61	17.87 ± 1.35	18.28 ± 2.10	23.24 ± 1.12	23.29 ± 0.80	19.45 ± 1.06	17.83 ± 0.87	19.84 ± 0.74
*Total Body Length	50.66 ± 0.25	47.46 ± 0.69	37.63 ± 4.66	44.28 ± 3.66	50.46 ± 2.83	48.83 ± 2.08	44.86 ± 1.73	41.41 ± 2.45	45.05 ± 1.20
*Shank Length (left side)	6.08 ± 0.02	5.66 ± 0.23	2.86 ± 0.97	5.45 ± 0.23	6.64 ± 0.23	6.04 ± 0.32	4.04 ± 1.01	4.02 ± 0.56	5.45 ± 0.24
*Shank Length (right side)	6.07 ± 0.03	5.86 ± 0.19	3.65 ± 0.67	5.04 ± 0.55	6.63 ± 0.25	6.03 ± 0.31	4.23 ± 1.07	4.05 ± 0.55	5.26 ± 0.19
*Phalanges Length (left side)	9.26 ± 0.19	8.30 ± 0.18	2.61 ± 1.61	6.06 ± 0.45	8.22 ± 0.38	7.42 ± 1.40	5.43 ± 1.37	5.86 ± 0.58	7.66 ± 0.24
*Phalanges Length (right side)	9.06 ± 0.31	8.27 ± 0.20	3.83 ± 1.57	6.44 ± 0.51	8.22 ± 0.38	7.82 ± 1.02	5.64 ± 1.44	5.83 ± 0.58	7.44 ± 0.24

B1= 0.2ml of 10 µg/mL mangosteen crude extract, using 50%ETOH
B2 =0.2ml of 50 µg/mL mangosteen crude extract, using 50%ETOH
B3 =0.2ml of 100 µg/mL mangosteen crude extract, using 50%ETOH

BR1= 0.2ml of 10 µg/mL mangosteen crude extract, using 50%ETOH
BR2 =0.2ml of 50 µg/mL mangosteen crude extract, using 50%ETOH
BR3 =0.2ml of 100 µg/mL mangosteen crude extract, using 50%ETOH

Figure 2. Shows the observed abnormalities occurred on 14-day old duck embryos after two weeks of incubation. (a) and (b) belongs to the negative control group, the NT and PBS group respectively. Embryos under this control showed no morphological abnormalities. (c) and (d) are duck embryos under the positive control (RA) group. (c) Contains blood clot all over its body, its mandible (lower beak) was thickened and malformed, it has open brain, flat eyes and microcephaly. (d) has malformed forelimbs, microcephaly, microphthalmia and its visceral organs were exposed. (e) A1, (f) A2 and (g) A3

are duck embryos treated with 10µg/mL, 50µg/mL and 100µg/mL mangosteen crude extract, using 95% ETOH, respectively. (h) B1, (i) B2 and (j) B3 are sample embryos treated with 10µg/mL, 50µg/mL and 100µg/mL mangosteen crude extract, using 50% ETOH, respectively. From (e) to (j), there was no abnormalities observed. Duck embryos from (k) to (p) are samples from the group treated with mixed retinoic acid and mangosteen crude extract, using 95% ETOH. (k) and (l) belongs to AR1 group; (m) and (n) are from AR2 group; while (o) and (p) are duck embryos under AR3 group. Sample embryos from (q) to (v) are samples from the group treated with mixed retinoic acid and mangosteen crude extract, using 50% ETOH. (q) and (r) belongs to BR1 group; (s) and (t) are from BR2 group; while (u) and (v) are duck embryos under BR3 group. Most of the abnormalities found in RA group were also seen on treated groups with mixed mangosteen crude extract and retinoic acid. However, as the concentration of extract in each set-up increases, the observed number of abnormalities and malformalities decreases. . (H: heart, S: stomach, R: ribs, SC: spinal cord). Scale bar = 10mm.

Culmen Length

The negative control and some of the treated groups showed significant values (P=0.000) when compared to the RA group. However the groups AR1(p=0.156), BR1 (p=0.444), BR2 (p=0.259) and BR3 (0.036) showed non significant value. These suggest that AR1, BR1, BR2 and BR3 have values related to the RA group.

Culmen Depth

In comparing the culmen depth of the treated groups, the following have shown significant values: NT (p=0.009), A2 (p=0.002), A3 (p=0.007), B1 (p=0.009), AR2 (p=0.009) when compared to the RA group.

Others that showed significant values were BR1 (p=0.006) when compared to the NT group; A2 (p=0.002), A3 (p=0.005), B1 (p=0.006) and AR1 (p=0.007) when compared to the group BR1.

Neck Length

Results showed there were no significant values on the compared neck length of the most treated groups and NT groups to the RA group. Some groups like A1 (p=0.003) and A3 (p=0.001) were significant when compared to the RA group.

Radio and Ulna Length

Most of the treated and control group shows no significant values in comparing the left side length of the duck embryos radio and ulna , except from the group AR3 (p=0.002) when compared to the RA group.

Gathered results from the right side length of the duck embryos radio and ulna was different from the results computed on the left side of the sample embryos. Significant figures were seen on the groups NT,A1, B1, B2 (having p= 0.006) and AR2 (p=0.001) when compared to the RA group.

Wing Length

As shown in the results, significant values were seen on the groups NT (p=0.002), PBS (p=0.008), A2 (p=0.009), A3 (p=0.001) and AR3 (p=0.003) when compared to the RA group. Other group that has significant figure on the left side of the wing length were NT (p=0.004), A3 (p=0.003) and AR3 (p=0.005) when compared to the BR1 group.

In comparing the right side length of the duck embryos wings, the following groups showed significant values when compared o the RA group: NT (p=0.001), A1 (p=0.000), A2 (p=0.002), A3 (p=0.000) and AR3 (p=0.003).

Body Length

There were significant values found on the groups A1 (p=0.000), A2 (p=0.001) and A3 (p=0.000), when they were compared to the RA group.

RA groups did not show significant values when compared to the groups AR1, AR2, AR3, BR1, BR2, and BR3. These may suggest that mangosteen crude extract (both extracts, using 95% and 50% ETOH) have not counter act the effect of retinoic acid on the treated duck embryos body length.

Total Body Length

The groups of A1, A2, A3 (all have p=0.000) and AR2 (p=0.005), showed significant values in comparing the total body length of the duck embryos of RA group to the other treated groups.

Shank (Torso metatarsus) Length

The following groups have showed significant values when compared to the RA group: NT (p=0.000), PBS (p=0.004), A1, A2, A3 (p=0.000), B2 (p=0.004), AR2, AR3 (both have p=0.000) and BR3 (p=0.10).

There are two groups showing significant values when the ducks right side length was compared to the RA group, the group of A2 (p=0.007) and AR2 (p=0.002) group.

Phalanges Length

Negative control group and most of the treated groups showed significant values when compared to the left side of the phalanges length of RA group. However, the groups AR1 (p=0.074), BR1 (p=0.292) and BR2 (p=0.120) showed non significant numbers.

Significant values were seen in the control group and most of the treated groups when comparing the right side length of the duck embryos phalanges under the RA group. However, other groups like B1 (p=0.016), AR2 (p=0.343), AR3 (p=0.010), BR1 (p=0.866), BR2 (p=0.760) and BR3 (p=0.032) did not show significant values.

Parts	Abnormalities	No. of Replicates	Negative Control		Positive Control	Treatments					
			NT	PBS	RA	AR1	AR2	AR3	BR1	BR2	BR3
Head	Microcephaly	5	0	0	5	2	0	0	4	3	1
	Open brain	5	0	0	1	2	0	0	3	0	0
	Enlarged cerebrum	5	0	0	2	2	4	2	2	3	3
	Enlarged midbrain	5	0	0	0	1	3	2	1	1	1
	Flat	5	0	0	2	3	1	1	4	1	0
Eyes	Hypertelorism	5	0	0	4	0	0	0	1	1	0
	Depigmented eye	5	0	0	2	3	3	2	3	3	1
	Micropthalmia	5	0	0	3	1	0	0	1	1	0
	No eyelids	5	0	0	2	0	0	0	2	1	0
Beak	Curve beak	5	0	0	3	3	2	1	5	2	1
	Short	5	0	0	3	3	0	0	3	3	2
Neck	Short	5	0	0	5	2	3	2	3	3	4
	Longer than the normal	5	0	0	0	1	1	1	0	0	0
Forelimbs	Short	5	0	0	2	2	0	0	2	2	1
	Malformed	5	0	0	3	3	2	0	3	2	0
	Absence	5	0	0	2	0	0	0	0	0	0
Hind limbs	Short	5	0	0	3	2	0	0	2	3	0
	Malformed	5	0	0	2	3	1	0	3	1	0
	Absence	5	0	0	2	0	0	0	1	0	0
Body	Un proportion	5	0	0	3	3	0	0	2	2	1
	Exposed visceral organs	5	0	0	2	1	0	0	3	0	0
Others	Blood clot	5	0	0	3	2	3	3	3	4	4
	Swollen body	5	0	0	2	2	3	1	3	4	0
	White colored body	5	0	0	3	3	2	4	3	2	4
Percentage of Abnormalities = (no. of abnormalities occurred in a group / total no. of overall abnormalities present) x 100			0%	0%	96%	83%	50%	42%	96%	79%	46%

Table 2. Abnormality Occurrence Found on Treated 3-day old Duck Embryos After 2- weeks of Incubation

BR1= 0.2ml of 10 µg/mL mangosteen crude extract, using 50%ETOH
BR2 =0.2ml of 50 µg/mL mangosteen crude extract, using 50%ETOH
BR3 =0.2ml of 100 µg/mL mangosteen crude extract, using 50%ETOH

From the gathered results, various abnormalities were observed on the groups treated with retinoic acid. In duck embryo's head, presence of microcephaly, open brain, enlarged cerebrum and enlarged midbrain were observed. In the part of the embryos eye, it was observed that it contained flat eyes, hypertelorism, some have depigmented eye, micropthalmia and others have no eyelids. In the duck embryos beak, it was observed that some contains curve beak while other have short beak. Treated embryos with retinoic acid in this study contained short and deformed neck. Absence of the appendages was also observed. On the other hand, some of the treated embryos were seen to have unproportioned body, some have exposed visceral organs, others have omphalocele, several have blood clot on some parts of their body, few have swollen body and others were observed to have white colored body.

One of the six principles of Teratology, given by James G. Wilson states that, susceptibility to teratogenesis varies with the developmental stage at the time of exposure of a teratogenic agent to an adverse influence; and also, there are critical

periods of susceptibility to agents and organ systems affected by these agents. From the said principle, retinoic acid as a teratogen in this study, was subjected to the 3-day old duck embryos. As Maden and his colleagues had observed in their study of endogenous retinoic acid (RA) distribution in the chick embryo, they found out that RA is released from tissues, apparently at a rate proportional to its rate of synthesis, and that is rapidly metabolized by cells. RA generation begins at gastrulation stage of chick embryo at a very low level and it begins to increase as the chick embryo develops from stage 4/5 and by its early (1st) somite stages. In duck embryogenesis, this stage is equivalent to 33-34 hours of development. The abruptly increase of RA levels sustained the development of the hindbrain neuropithelium formation of the somites at the spinal part of the neural tubes and neural crest. In short, RA has a major role in the development of the central nervous system (CNS). Addition of foreign RA (in this study, 13-cis retinoic acid was used, also known as accutane) at the stage that developing cells is actively synthesizing RA (in the experiment, RA was injected to the 3-day/ 72 hrs duck embryo), theoretically, it will double the substance amount, making it to become toxic (Kochhar 2009).

Retinoic acid induced cell death has been shown to cause limb reduction, defects in mice (Alles and Sulik 1989). In this study, embryos treated with retinoic acid also showed limb reduction or deformation and absence of beak. In this study, cephalic disorders were also observed in the RA-treated embryos like microcephaly, open brain, and brain haemorrhage. Retinoic acid also causes abnormalities to neural crest cell derivatives the neural crest cells migrate throughout the embryo to give rise to many different cells of the peripheral nervous system, cartilage, pigment cells or melanocytes (Baroffio et al. 1990). Because the production of melanocytes

was affected, retinoic acid-treated embryos showed depigmentation of the eyes and body in this study.

The gross morphological abnormalities observed in the RA-treated embryos (positive control) compared with AR1, AR2, AR3, BR1, BR2 and BR3 treated embryos were used to gauge the level of effectiveness of mangosteen extract as an antiteratogen. Based from the results, reduction of duck embryos abnormalities were observed as the dosage of both mangosten crude extracts, extracted using 95% and 50% ETOH increases on treatments with retinoic acid. Reduction of malformalities in this study may indicate antiteratogenic activity (Assayed et. al 2010, Herrera et. al 2010 and San Diego 2008).

Treatment of the duck embryos with both mangosteen crude extracts, extracted using 95% and 50% ETOH alone did not result statistically significant numbers when compared to the negative control groups (both the NT and PBS groups), indicating that these extracts with the dosage of 10, 50, and 100µg/ml are nonteratogenic. On the other hand, when both the mangosteen crude extracts with mixed retinoic acid were tested, both group treatments showed decrease percentage of abnormalities as the dosage of the mangosteen crude extracts increases. These may indicate that the mangosteen crude extracts, extracted with 95% ETOH and 50%ETOH has an antiteratogenic property.

Based on statistical analysis of the results, this antiteratogenic activity seemed more effective in higher amount (50 µg/ml and 100µg/ml) of both the mangosteen crude extracts, extracted with 95% and 50%ETOH, than in the lower concentration (10 µg/ml). Those treated with AR1 and BR1 had an insignificant difference in mean frequency of measured morphological characters compared with RA-treated embryos. This implied that the small dosage of the extract is not sufficient.

Based on the findings, both the mangosteen crude extracts, extracted using 95% and 50% ETOH confered protection to embryo during its development. One possible chemical component of mangosteen crude extract that may have antiteratogenic action is the anti-oxidants. Garcinia mangostana contain 5.5% dry weight basis of the antioxidant phenolic acid, which may protect cell membranes against damage by oxygen radicals (Zadernowski 2009). Mangosteen also contains Vitamin C, which is about 12% of per 100 g of powdered fruit hull (http://www.nutrition-and-you.com/mangosteen.html). Vitamin C as an antiteratogen can decrease fetal malformation rate, diminish oxygen radical-related tissue damage, and ameliorate oxidative protein carbonylation in fetal livers as well as they can fully restore diabetes-induced lipid peroxidation and improve gestational outcome of a pregnant female Wistar rats (Assayed et al. 2010). The above mentioned antioxidants present in mangosteen fruit hulls, might have possibly given its observed antiteratogenity in this study. However further studies must be established in order it to be proven. Other major antioxidants in mangosteen are (Thiamine) B1, Protein, Iron, Calcium Fiber, Potassium, Phosphorus, Sodium, Niacin, and Polyphenols, Cathechins/tannins, Flavonoids, Succinate, glycerophosphate, ascorbate and xanthones (especially the α, ß and γ xanthones).

CONCLUSIONS

Based on the results obtained, the following were deduced (1.) 10, 50 and 100µg/ml of both mangosteen crude extract, extracted with 95% and 50% ETOH are not teratogenic; (2.) Mangosteen crude extracts have lessen the malformations brought by the teratogen retinoic acid on the duck embryo's head width, eye diameter, culmen length, culmen depth, radius and ulna length, wing length, body lenth, total body length,

shank length and foot length; (3.) Among the two mangosteen crude extracts tested, the dosage that seemed more effective was found on 100µg/ml; and (4.) Mangosteen crude extract, extracted using 95% ETOH has higher yield of altering malformations caused by retinoic acid than mangosteen crude extract, extracted using 50% ETOH.

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