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PRELIMINARY STUDY OF THE EFFECTS OF CALABASH (*CRESCENTIA CUJETE*) ETHANOLIC FRUIT EXTRACT TO GESTATING SPRAGUE DAWLEY RATS

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***Abstract*— It has been reported that the water fruit extract of Calabash (*Crescentia cujete)* have many traditional claims for herbal medicine. However the safety of its use during pregnancy has not been fully investigated. The ethanolic fruit extract (25%, 75%, and 100%) was given to Sprague Dawley rats on days 6 to 19 of gestation, that is, organogenetic period. Maternal organ and fetal weight were recorded; the morphometric indices such as Crown-rump length (CRL), Forelimb length (FLL), Head-lip length (HdLL), and Hind-limb length (HLL) of the fetuses were carried out. The Control group had the heaviest fetal weight, followed by 75%, 25% and 100% concentration. The Control Group ranged from 3.5 to 6.3g (mean = 5.07 ± 0.86). The 75% concentration group ranged 3 to 5.2 g (mean = 3.89 ± 0.55). The 25% concentration group ranged from 2.6 to 4.9 g (mean = 3.78 ± 0.49). The 100% concentration group ranged from 3 to 4.3 g (mean = 3.53 ± 0.36). No remarkable abnormality in the external morphology of fetus was observed other than the significantly reduced fetal weight. Blood samples were also collected and were analyzed for complete blood profile (CBP): red blood cell (RBC), white blood cell (WBC), platelet, hematocrit and hemoglobin. The blood count shows that the control group is within every expected range (with the exception of the 25% group in the WBC count, which exceeds the expected range of 5.0-10.0 x10^9/L). Continuous consumption of higher concentrations of the extract during gestation can alter the growth and development of the fetus and maternal organs and the maternal blood count. Utmost caution should be done by humans in using this extract during pregnancy.**

Index Terms— calabash, Crescentia cujete, gestation, Sprague dawley rats

I. INTRODUCTION

The use of plants for remedies has long been in existence and is among the most attractive sources for developing drugs [1]. Any part of plant can be considered as herbs including leaves roots, flower, seeds, resins, leaf sheath, bark, inner bark (cambium), berries and sometimes the pericarp or other portion [2]. Most primates depend heavily on the leaves, fruits, and flowers of tropical plants to meet their nutritional demands [3][4][5]. As a result, the chemical composition of these plant parts is critical to understanding primate ecology and evolution. These ancient indigenous practices were discovered by series of 'trial and error' which then could not be substantiated by proven scientific theories [2].

Fruits are rich with antioxidants that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species [6]. The most abundant antioxidants in fruits are polyphenols and vitamins C, A, B and E; while carotenoids are present to a lesser extent in some fruits. These polyphenols with antioxidant activities are mostly belong to flavonoids [7].

The Calabash tree, scientifically known as *Crescentia cujete*, has been used by indigenous system of medicine to treat several illnesses. The pulp of fruit has medicinal properties and acts as remedy for respiratory problems such as asthma and cough. The leaves are used to reduce blood pressure. The decoction of tree bark is used to clean wounds and also to treat hematomas and tumors. Because of this, Calabash tree is considered to be a miracle fruit [8].

On the other hand, according to Thewlis and Meyer [9] rats are becoming increasingly popular as small laboratory for hematologic studies, since blood is easily obtained in adequate quantities for routine counts. There is considerable fluctuation in the leukocytes particularly, but the instability seems to be no greater than that of guinea pigs or rabbits. Rats are less expensive than either, and blood is more easily obtainable from them than from guinea pigs [9].

Very few literatures have been explored as to the potential of its fruit pulp ethanolic extract for organogenesis in female rat vertebrate model and its overall hematological effects in gestating rats, as blood parameters are sensitive indicators to an organism's physiological state [10]. This study looks into the possible effects of *Crescentia cujete* ethanolic fruit pulp extract to the maternal-fetal development and growth of Sprague Dawley rats. The effect of the Calabash fruit ethanolic extract on the blood count of female Sprague Dawley rats during its gestating period is also evaluated in this study.

II. MATERIALS AND METHODS

The Calabash fruit (Fig. 1) was collected from Brgy. Ambago, Butuan City, Philippines. It was identified by the Department of Environment and Natural Resources (DENR)-CARAGA, Philippines as *Crescentia cujete*. Three concentrations of *C. cujete* extract were used in the study: 25%, 75% and 100%.

Sixteen female and male Sprague Dawley rats approximately 6 months old (weighing between 140 to 160g) and of proven fertility, were subjected to experimental analysis. Animals were completely randomized into 4 groups of 4 rats each. Group I (control) was given distilled water, while groups II, III and IV were given 25%, 75% and 100% *C. cujete* ethanolic fruit extract orally from days 6 to 19 of gestation, that is, their organogenetic period. Rats were then sacrificed on day 19 of gestation, and the uteri of all the sacrificed rats were examined. Body weight, diet consumption and water intake of all the groups were recorded daily. The rat's fetuses were also individually weighed and morphometrically measured in terms of their crown-rump length (CRL), forelimb length (FLL), head-lip length (HdLL), and hind-limb (HLL) length. Lastly,

the collected blood samples were analyzed for complete blood profile (CBP): red blood cell (RBC), white blood cell (WBC), platelet, hematocrit and hemoglobin. These laboratory procedures were conducted in Father Saturnino Urios University College Science Laboratories.

III. RESULTS AND DISCUSSION

A. Mortality and Gross Morphologic and Fetal Observations

The experiment was terminated on the 19th day of gestation (GD 19). Within the first 5 gestating days, the rats had an abrupt gain of weight and increased in appetite were observed as a result of breeding. It showed that rats treated with 100% of the fruit extract were significantly (P>0.05) heavier than those treated with lower concentrations and the control (Fig. 2, Fig. 3). The weight and size of the fetus of the experimented rats also confirms the result of *C. cujete* extract effect on body weight. The exact reason for this observation is not too clear.

In terms of fetal weight, the Control group had the heaviest fetal weight, followed by 75%, 25% and 100% concentration (Table I, Fig. 4). The Control Group ranged from 3.5 to 6.3g (mean = 5.07 ± 0.86). The 75% concentration group ranged 3 to 5.2 g (mean = 3.89 ± 0.55). The 25% concentration group ranged from 2.6 to 4.9 g (mean = 3.78 ± 0.49). The 100% concentration group ranged from 3 to 4.3 g (mean = 3.53 ± 0.36). Morphological examination (Fig. 5) was also done on the rats' fetuses and no remarkable abnormality in the external morphology of fetus was observed other than the significantly reduced fetal weight (Fig. 6).

Fig. 1. **Calabash (*Crescentia cujete*) collected from Brgy. Ambago, Butuan City, Philippines.**

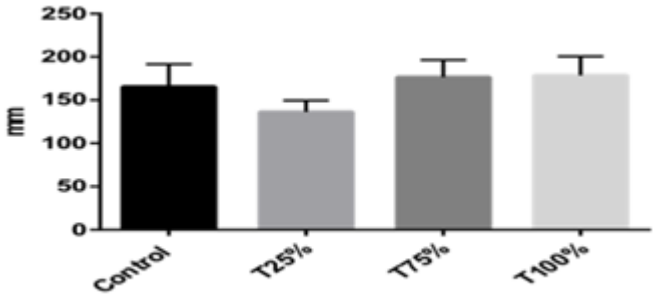

Fig. 2. **Overall Relative Weight in grams of Gestating Sprague Dawley rats from GD 0 to GD 19.**

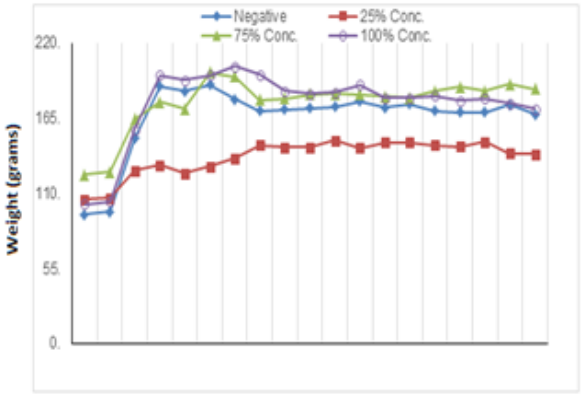

Fig. 3. **Overall Relative Weight in grams of Gestating Sprague Dawley rats from GD 0 to GD 19.**

TABLE I. SUMMARY OF PARAMETERS MEASURED IN THE RAT FETUS TREATED WITH *C. CUJETE* FRUIT ETHANOLIC EXTRACT.

Treatment	Fetal weight (g)	CRL (mm)	FLL (mm)	HdLL (mm)	HLL (mm)
Control	5.07	38.23	15.17	15.33	15.28
25%	3.78	32.91	12.93	17.82	17.31
75%	3.89	32.02	12.45	13.74	13.35
100%	3.53	30.69	12.38	13.29	13.04

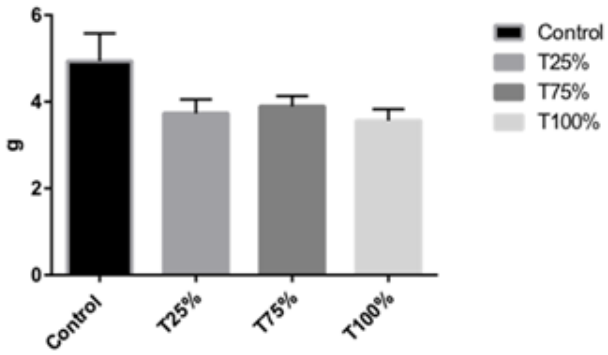

Fig. 4. **Relative fetal weights exposed from different concentration of *C. cujete* fruit ethanolic extract.**

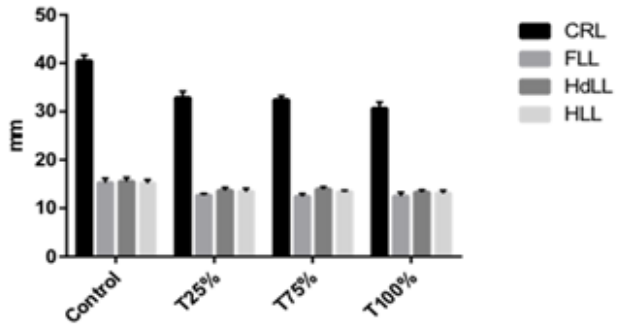

Fig. 5. **Morphometric fetal indices of Sprague Dawley rats exposed in different concentration to *C. cujete* fruit extract. CRL- Crown rump length, HdLL- head lip length, FLL- forlimb length, HLL-Hindlimb length.**

Fig. 6. **GD 19 Sprague Dawley fetuses exposed *in utero* to *C. cujete* ethanolic fruit extract. (A,E,I) Control; (B,F,J) fetus exposed in 25% concentration of *C. cujete* fruit ethanolic fruit extract. (C,G,K) foetus exposed in 75% concentration of *C. cujete* fruit ethanolic fruit extract. (D,H,L) foetus exposed in 100% concentration of *C. cujete* fruit ethanolic fruit extract.**

B. Hematology

The result of the haematocrit test is presented in Table II. The control has an average result of 0.44 ± 0.40. The 25% concentration shows significant drop below the expected range, with an average result of 0.20 ± 0.10. In the white blood cell test, the negative control has an average result of 7.34 ± 5.10. The 25% concentration however, is slightly above the expected range, with an average result of 13.60 ± 12.30. The platelet test shows that the control is within the expected range of 150-390 x10^9/L. It has an average result of 210 ± 180. The 25%, 75% and 100% however, is relatively lower than the control.

IV. DISCUSSION

The abrupt weight gain of the Sprague Dawley rats was because of their pregnancy. According to Stoppard [11] in her book "Conception, Pregnancy and Birth, it is normal that in the first part of gestation, there is an increase in appetite. The Ghrelin, a hormone that is released primarily in the stomach and is thought to signal hunger to the brain increases during pregnancy [12]. The sudden increase in weight of the mothers can also be attributed to the developing embryo. The *C. cujete* is known to possess Hydrogen Cyanide. The mean value of the hydrogen cyanide in the *C. cujete* fruit was found to be cyanide value of 0.01mg/L as defined by WHO [8]. This suggests that the continual consumption of *C. cujete* fruit extract may

TABLE II. BLOOD COUNT RESULTS AFTER GD 19 TREATMENT VALUES ARE MEAN ± SD FOR 4 TREATMENTS HAVING 3 RATS IN EACH REPLICATES.

Treatment	Hemoglobin	Hemo Mean	Hematocrit	Hema Mean	White blood cell	WBC Mean	Platelet	Plate Mean
Control	146.00 ± 133.00	139.67	0.44 ± 0.40	0.420	7.34±5.10	6.213	210 ±180	195.00
25%	33.00 ± 31.00	32.00	0.20 ± 0.10	0.137	13.60±12.30	13.100	40 ±36	38.00
75%	137.00 ± 115.00	124.00	0.41 ± 0.38	0.397	5.60±2.26	3.670	50 ±37	45.00
100%	135.00 ± 121.00	129.67	0.40±0.36	0.380	2.59±1.39	1.990	122±15	52.33

eventually lead to hydrogen cyanide toxicity. It is a chemical asphyxiant; it stops the tissue from utilizing oxygen which makes it a potential fatal poison [12].

The relative morphometric measurements revealed that the rat fetus at 100% Calabash ethanolic extract has the shortest in head-lip length, crown-rump length, forelimb length and hind limb length and the lightest in weight. While the heaviest and the longest in all measures was yielded by the mildest concentration (25% concentration). These can also be attributed to possible hypothyroidism in the gestating mother rats as caused by the high demand of iodine during pregnancy and the inducement of the fruit extract containing hydrogen cyanide that impairs iodine uptake in the thyroid gland [13].

In this study, the significant drop of the over-all blood count of Sprague Dawley rats from their expected ranges may have been caused by the recorded phytochemical constituents of the Calabash fruit referred from the team of Ejelonu [8] in Nigeria. The plant is considered to have alkaloids, saponins, flavonoids, anthroquinone, cardenolides, tannins and phenols. Even though phytochemicals are beneficial, plant alkaloids, tannins and phenols are also considered to be toxic at a definite level and considered to be corrosive [14]. In other words, it is possible that the level of the phytochemical constituents of calabash is high enough that it exhibits cytotoxicity which is evident on the blood count results of the gestating Sprague Dawley rats.

From the observed values of WBC, it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alter their normal physiological processes. The leucocytosis observed in the present study indicates a stimulation of the immune system which protects the rats against infection that might have been caused by chemical and secondary infections. Leucocytosis, which may be directly proportional to the severity of the causative stress condition, may be attributed to an increase in leukocyte mobilization [15].

V. CONCLUSION

Although the Calabash (*C. cujete*) ethanolic fruit extract contains essential secondary plant metabolites that can treat various health problems, the continuous consumption of the extract at gestation period which is evident in the animal model, shows that the higher concentrations of the ethanolic fruit extract can alter the growth and development of the fetus and alters the blood profile of the maternal rats. Therefore, with utmost caution, should be advised in the use of this extracts during pregnancy in human.

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