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EFFECT OF ADMINISTRATION OF FLAVONOID-RICH EXTRACT OF *HIBISCUS SABDARIFFA* ON PLASMA GLUCOCORTICOID LEVEL ON PREGNANT RATS

Anthonia Onyinye Ngwoke[1], Tobechukwu Chigha Iyidobi[2] and Emmanuel Ifeanyi Obeagu[3]*

[1]Department of Physiology, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria.

[2]Department of Medicine, University of Nigeria Teaching Hospital Ituku/Ozalla, Enugu, Nigeria, ORCID: 0000-0003-4237-6084.

[3]Department of Biomedical and Laboratory Science, Africa University, Zimbabwe.

***Corresponding Author: Emmanuel Ifeanyi Obeagu**

Department of Biomedical and Laboratory Science, Africa University, Zimbabwe.

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ABSTRACT

The nutritional status of a woman before and during pregnancy is important for a healthy pregnancy outcome . This study investigated the effect of flavonoid-rich extract of *Hibiscus Sabdariffa* on plasma glucocorticoid level of pregnant Wistar rats. 20 female Wistar rats weighing 120-220g was used for this study. They were divided into control, low dose group (given 10mg/kg of flavonoid rich extract), medium dose group (given 20mg/kg of flavonoid rich extract), and high dose group (given 50mg/kg of flavonoid rich extract). There was a significant increase in corticosterone. The use of *flavonoid-rich* extract by the mother does not have a toxic effect on the offspring hence, consumption of flavonoids as supplement should be encouraged among pregnant women to enable them shed weight (weight loss) that resulted from fat accumulation during pregnancy.

KEYWORDS: Flavonoid, Hibiscus, Sabdariffa, Glucocorticoid, Pregnancy.

INTRODUCTION

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages. Many of them have been shown to have free radical scavenging capacity, coronary heart disease preventive, hepatoprotective, anti-inflammatory, anticancer, antiviral and antioxidative activities.[1] *Hibiscus sabdariffa* which belongs to the Malvaceae family is an annual shrub with several branches, relatively found in different parts of the world especially in Malaysia, Thailand, India, and tropical Africa including Nigeria.[2-3] A wide range of studies had reported that the extracts from Hibiscus sabdariffa calyces possess different bioactive constituents which flavonoid is one of them.[4] The nutritional benefits of the plant are associated with the bioavailability of flavonoids, particularly anthocyanins.[5] Extracts of *HS* are widely used in folk medicine for the treatment of a variety of ailments such as hypertension, hepatic disorders and febrile conditions.[6]

The Fresh or dried calyces are also used in the preparation of herbal drinks, hot and cold beverages, fermented drinks, wine, jam, jellied confectionaries, ice cream, chocolates, flavouring agents, puddings and cakes.[7-8]

Report from[9] shows that consumption of aqueous extract of *HS* by pregnant rats decreased litter Size and increased litter birth weight in their offspring; and the increased litter birth weight in spite of the decreased maternal food consumption implies a fetal growth promoting effect of the *HS* extract. The decreased litter size is due to an anti-implantation effect of aqueous extracts from HS, by causing biochemical and biophysical alterations in the endometrium.[10] The placenta is the organ responsible for the transfer of nutrients from mother to the fetus. The placenta therefore can respond to maternal environmental stimuli which will affect the growing fetus.[11] Studies have shown that exposure to elevated glucocorticoids during pregnancy has been associated with adult onset diseases like; hypertension, cardiovascular pathologies, altered metabolic functions and other pregnancy related conditions like intra-uterine growth restriction(IUGR), Preterm birth and preeclampsia.[12] Also, elevated glucocorticoids have been shown to inhibit implantation by causing inadequate biochemical differentiation at implantation sites.[13] It also causes complete abortion and resumption at implantation sites. Meanwhile,

consumption of aqueous extract of HS by pregnant rats resulted to an increased plasma glucocorticoid concentration.[14] It is not clear if the effects observed due to consumption of aqueous extract of HS by pregnant rats are due to its flavonoid component. It is not also clear if the elevated glucocorticoid level due to extract from *HS* is due to the flavonoid it contains.

MATERIALS AND METHOD

PLANT COLLECTION AND IDENTIFICATION

Matured dried red calyces of *Hibiscus sabdariffa* was purchased from Ogbete main market in Enugu State, Nigeria. It was identified and authenticated by a taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A sample was kept in the university herbarium for reference purposes.

PREPARATION OF THE EXTRACT

The extraction procedure was according to the method of Iyare and Adegoke.[15] A dried calyx of HS was crushed and 2000g of the calyces powder were extracted by maceration in 5L of methanol for 48h with intermittent agitation. The powder was washed repeatedly with fresh portions of methanol to ensure an exhaustive extraction. The methanol extract was recovered by evaporation of the filtrate in a vacuum at 40°C using a rotary evaporator. The concentrated extract were transferred into sterile beakers, covered with aluminum foil and stored in a refrigerator. The extracts were screened for the presence of different phytochemicals by employing the standard methods of Trease and Evans. The constituents found in the extracts were alkaloids, saponins, glycosides, flavonoids, phenols, tannins, steroids and reducing sugars.

PREPARATION OF FLAVONOID-RICH EXTRACT

The flavonoid-rich extract of HS was prepared following the methods of Chu et al., (2002) with slight modification: A 100g of the crude extract was dissolved in 500ml of 10% H2SO4 in a conical flask and hydrolyzed by heating on a water bath for 30minutes at 100°C. The mixture was then placed on ice for 15minutes to allow the precipitation of the flavonoid aglycones. The cooled solution was filtered and the filtrate(flavonoid aglycone mixture) was dissolved in warm 95% ethanol. The resulting solution was filtered again into a volumetric flask. The filtrate will be concentrated to dryness using a rotary evaporator. Test for flavonoid was performed to confirm the preparation. To a portion of the extract, a few drops of 1% aluminium solution were added, a yellow coloration after few minutes indicated the presence of flavonoids. The extract was stored under 40°C before use and reconstituted in distilled water to give required doses of flavonoid. The doses were prepared fresh on the day of experiment prior to administration to the rats.

EXPERIMENTAL ANIMALS

Twenty in-bred virgin female Sprague -Dawley rats weighing about 150g were used for this study. These rats were obtained from animal house of the faculty of Basic Medical science, University of Nigeria, Enugu Campus and housed in plastic cages. The animals were allowed to acclimatize for 7 days under standard laboratory conditions with free access to chow and water.

INDUCTION OF PREGNANCY IN RATS

The rats estrous cycles was monitored daily under light microscopy and male Wister rats of proven fertility were introduced into the cages of the female rats that will be expected to get into the estrous phase within 12 hours to allow mating in the ratio of 1 male to 2 female rats. The day that sperm was observed in the vaginal smear of the female rats was taken as day 1 of pregnancy.[15] Animals were then assigned to their various groups.

EXPERIMENTAL DESIGN

On day 1 of pregnancy, animals were divided randomly into four groups of five animals each.

Group A is the control group and received only chow and water throughout pregnancy.

Group B - In addition to normal chow and water, received Low dose of 10mg/kg of flavonoid-rich extract.

Group C- In addition to normal chow and water, received Low dose of 20mg/kg of flavonoid-rich extract.

Group D - In addition to normal chow and water, received Low dose of 50mg/kg of flavonoid-rich extract.

Administration was by oral gavage and commenced on day 1 of pregnancy and ended on day 18.

MEASUREMENT OF FOOD INTAKE

The quantity of food being administered was weighed and recorded daily. Before administration of the next meal, the leftover was gathered and recorded and then subtracted from the original quantity that was administered the previous day and then recorded.

MEASUREMENT OF FLUID INTAKE

All the animals in the various groups were fed daily with water. Fluid intake was calculated by subtracting the quantity remaining from the original amount of fluid given the previous day.

MEASUREMENT OF PREGNANCY WEIGHT GAIN

A digital electronic weighing scale was used to determine the weight of dams weekly.

BIOCHEMICAL ANALYSIS

COLLECTION OF BLOOD SAMPLE

This procedure was carried out according to the method described by Iyare et al. (2010). On the 18th day of gestational, the rats in the different groups were anaesthetized by chloroform inhalation. The thoracic cavity was quickly opened and the heart exposed. Blood sample was withdrawn by cardiac puncture using a 19G hypodermic needle. The needle was removed and the

blood ejected into a heparin-containing specimen bottle. The blood was immediately spunned at 3000 rpm for 10 minutes in a centrifuge. The plasma was gently withdrawn using a Pasteur pipette and stored at 20°C.

DETERMINATION OF PLASMA GLUCOCORTICOID

The rat corticosterone Elisa kit was used for the determination of the blood glucocorticoid level.

PROCEDURE

All reagents where first brought to room temperature (18-25ºC) before preparation and use. 50µL concentration of the standard working solution was added to each well on the first two columns. Immediately afterwards, 50µl of Biotinylated Detection Ab working solution was added to each well. The plate was covered with a sealer and then incubated for 45 minutes at 37ºC The solution was then decanted or aspirated from each well and then 350µL of wash buffer was added to each well. After about 2 minutes, it was decanted and dried against a paper absorbent. This step was repeated 3 times 100µL of HRP Conjugate working solution was then added to each well, and then covered with the plate sealer and incubated for 15 minutes at the 37ºC. It was then aspirated again and the wash process was repeated five times just as it was done in step four. 90µL of the substrate reagent was then added to each well. A new plate sealer was used to cover the wells and incubated for 15 minutes at 37ºC while being protected from light. This produced a colour change from blue to yellow. 50µl of stop solution was then added to each well. The optical density of each well was then read off on the ELISA machine using a microplate reader set to 450nm.

MEASUREMENT OF FETAL AND PLACENTAL WEIGHTS

After collection of blood samples, the gravid uteri were removed with their contents. The fetal weights were determined using electronic weighing scale. The placentas were carefully removed and also weighed.

PLACENTAL HISTOLOGY

The placentas of the control and flavonoid treated rat groups were histologically examined. After carefully removing them, the placentas were fixed in 10% buffered formaldehyde. Sections were obtained and stained with haemotoxylin and eosin (H & E) stains. The microscopic slides were labelled appropriately and photomicrograghs taken at magnification of X100 using a light microscope.

STATISTICAL ANALYSIS

All data were tabulated and statistically analyzed. Results were presented as mean ± standard error of mean. The results were analyzed using one way analysis of variance (ANOVA), followed by student Newman-keul's post-hoc test. A value of $P< 0.05$ was taken as statistically significant. All the analyses were carried out using the statistical package for social science (SPSS) Version 25.0 analytical software for Windows.

ETHICAL APPROVAL

The ethical clearance for this study was obtained from the research and ethics committee of the College of Medicine, University of Nigeria, Enugu Campus.

RESULTS

Table 1: Effect of Maternal Consumption of Flavonoid During Pregnancy On Maternal Plasma Corticosterone (Ng/Dl).

GROUPS	PLASMA CORTICOSTERONE (ng/dl)
CONTROL(food+water)	0.22±0.00
LOW DOSE (10mg/kg flavonoid)	0.34±0.00[a]
MEDIUM DOSE (20mg/kg flavonoid)	0.31±0.00[a]
HIGH DOSE (50mg/kg flavonoid)	0.34±0.13[a]

Each value represents mean ± SEM, n=4, [a]=p<0.05vs Control.

DISCUSSION

The observed increase in the plasma corticosterone in this study is affirming the report of Iyare[14] that "consumption of aqueous extract of *HS* by pregnant rats resulted to an increased plasma glucocorticoid concentration. The flavonoids in the *HS* extract could have inhibited the activity of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD-2).[16-17] This greatly reduces the conversion of the active glucocorticoid to the inactive form and thus increases the level of glucocorticoid. The observed reduction in litter size when compared with that of the control could be as a result of the elevated glucocorticoid (corticosterone) level because elevated glucocorticoids have been shown to inhibit implantation by causing inadequate biochemical differentiation at implantation sites.[13] It also causes complete abortion and resumption at implantation sites.[18]

CONCLUSION

In conclusion, this study showed clearly that maternal consumption of flavonoid-rich extract from *HS* plant has a reducing effect on the body weight and food intake of

dams during pregnancy but no adverse effect on the fetal weight and placental weight.

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