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EFFECTS OF *HIBISCUS SABDARIFFA* L. CONSUMPTION DURING PREGNANCY ON BIOCHEMICAL PARAMETERS AND LITTER BIRTH WEIGHT IN RATS

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Abstract

Background: Extracts of *Hibiscus sabdariffa* L. (HS; family: Malvaceae) are widely believed in folk medicine to be effective in the treatment of a variety of ailments. In Nigeria, anecdotal reports by women who consume HS during pregnancy suggest that they consume it because of the folkloric belief that it makes them "feel lighter". This study aimed to investigate the effect of maternal consumption of HS during pregnancy on litter birth weight and the functional integrity of the liver of pregnant rats.
Materials and Methods: Thirty-six rats aged twelve to fourteen weeks were used. On day 1 of pregnancy, the rats were randomly assigned to three groups. Group A were given tap water, group B were given 0.6g/100ml while group C were given 1.8g/100ml of the extract. On day 18 of pregnancy, blood samples were taken for determination of Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase, urea and creatinine.
Results: Gestational length, litter size and birth weights were recorded at delivery. HS consumption did not affect ($P>0.05$) SGOT level but increased ($P<0.05$) the SGPT and decreased ($P<0.05$) ALP and creatinine levels. The low dose HS decreased ($P<0.05$) while the high dose HS increased ($P<0.05$) the urea level. There was no significant ($P>0.05$) difference in the length of gestation but litter sizes and litter birth weights were significantly ($P<0.05$) lower and higher respectively when compared with the control.
Conclusion: We conclude that consumption of HS during pregnancy increases litter birth weight possibly through the gluconeogenic activities of the elevated SGPT.

Key words: SGOT, SGPT, Urea, Creatinine, Birth weight, *Hibiscus sabdariffa*

Introduction

There is an upsurge in the use of herbal remedies for the management of a variety of ailments because of the erroneous belief that they are harmless, since they are unrefined natural products. Most of these herbal decoctions are commonly produced and sold and are not scientifically tested. The modes of preparation (and hence the concentrations) differ - they are very cheap (compared with non-herbal medications) and their sale and uses are not regulated. Some of these herbal remedies are sweetened (because they have unpalatable taste) while others are not.

Hibiscus sabdariffa (HS; family: Malvaceae), commonly called 'zobo' in Nigeria, is an annual, erect, bushy, herbaceous sub-shrub that grows up to 8 ft. (2.4 m) tall, with smooth or nearly smooth and cylindrical stem. Extracts of HS are widely believed in folk medicine to be effective in the treatment of a variety of ailments (Oliver, 1960; Perry, 1980). The effectiveness of HS in the treatment of these ailments has been attributable to its various constituents like phytochemicals - flavonoids, anthocyanin etc., (Fuleki and Francis, 1968; Duke and Francis, 1973; Clydesdale, 1979; Morton, 1987; Daffalah and al-Mustafa, 1996; Appel, 2003; Adigun et al., 2006).

In Nigeria, a sweetened aqueous extract of HS (zobo drink) is commonly produced, sold and consumed. Some women have even been observed consuming zobo drink during pregnancy. Anecdotal reports by these women suggest that they consume it because of the folkloric belief that it has haematopoietic properties and that it also makes them "feel lighter".

The liver is an important organ in the body that plays a vital role in the metabolism of foreign compounds entering the body. Serum glutamate oxaloacetate transaminase (SGOT) or aspartate transaminase (AST) and serum glutamate pyruvate transaminase (SGPT) Alanine transaminase (ALT) are the most important enzymes of the liver parenchyma cells. They are in large numbers in the liver and are released in the blood when the liver cells get damaged. There are several reports on the hepatoprotective effect of aqueous extract of *Hibiscus sabdariffa* against several experimentally induced hepatotoxicity (Wang et al., 2000; Liu et al., 2002; Ali et al., 2003; Amin and Hamza, 2005; Asagba et al., 2007; Olaleye and Rocha, 2008).

There is paucity of data on the effect of maternal consumption of aqueous extract of HS during pregnancy on birth weight and the liver of normal pregnant rats. This study was designed to investigate this.

Materials and Method

Animals

Thirty-six (36) inbred virgin female Sprague-Dawley rats aged between twelve (12) and fourteen (14) weeks with two consecutive regular four-day estrus cycles and weighing 110-130g were used for this study. These rats were obtained from the animal house of the Department of Physiology, College of medicine, University of Nigeria, Enugu Campus.

The estrus cycles were monitored for each rat by examining, under light microscopy, the daily vaginal smears. At proestrus, male rats of proven fertility were introduced into the female cages to allow for mating. Mating was deemed successful when spermatozoa were observed

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in the vaginal smear of the female rats the following morning. The day spermatozoa were observed in the vaginal smear was regarded as day 1 of pregnancy (Mallie and Boudzoumou, 1996). On day 1 of pregnancy, the rats were randomly assigned to one of three treatment groups: A, B and C. Group A rats were given tap water only, group B rats were given 0.6g/100ml of the extract while group C rats were given 1.8g/100ml of the extract. On day 18 of pregnancy, blood samples were withdrawn from six rats per group according to the method of Raji et al. (2005) for the determination of the biochemical parameters. Gestational length, litter size and birth weights were recorded at delivery.

All procedures used in this study conformed with the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002) and were approved by the Departmental Committee on the Use and Care of Animals.

Extraction Procedure

Matured dry dark-red calyces of HS were purchased from a local market in Enugu, Nigeria and authenticated by a botanist in the Department of Pharmacognosy, University of Nigeria, Nsukka. The extraction procedure used was as described previously (Iyare et al., 2010a, b). Briefly, the dry calyx of HS (30 g) was brewed in 400 ml of boiled tap water for 45 min. The resulting decoction was filtered using filtration sieve and the filtrate was evaporated to dryness giving a dark red powder (yield 48.87 %). This dark red powder was stored in a refrigerator until needed for the preparation of the respective drinking solutions.

When needed, 0.6 g and 1.8 g of the dark red powder were then weighed and dissolved in 100 ml of tap water and given to the respective groups of rats as their drinking solutions.

Determination of Biochemical Parameters

The withdrawn blood sample was put in a heparinized tube and spun in a centrifuge at 3000rpm for 15 minutes to obtain the plasma. The plasma was then used for the determination of SGOT, SGPT, Alkaline phosphatase, urea and creatinine using the established methods described in the assay kits (Randox England).

Statistical Analysis

Results are expressed as mean ± standard error of mean (M±SEM). For data comparison, the one way analysis of variance (ANOVA) was used followed by a post-hoc Student's Newman-Keuls test. $P < 0.05$ was taken as statistically significant. All the analyses were carried out using the SPSS Version 15.0 for Windows.

Results

Pregnancy Weight Changes

There was no significant difference (P>0.05) in the pregravid weights among the rats in the three groups but there was a statistically significant reduction (P<0.05) in term weight and weight gain (term weight – pregravid weight) in the HS groups compared with the control group.

Table 1: Effect of consumption of aqueous extract of *Hibiscus sabdariffa* L. during pregnancy on pregnancy weight gain

Groups	Pregravid weight(g)	Term weight (g)	Weight gain(g)
Control	124.36±3.73	227.5±5.4	103.13±2.37
0.6g/100ml	122.5±8.05	211.67±6.28*	89.17±2.20*
1.8g/100ml	122.5±2.3	202.5±4.5*	80.0±5.1*

N = 6 each. Values are expressed as Mean ± SEM, * = P<0.05 vs Control.

Biochemical Parameters

HS consumption did not significantly (P>0.05) affect SGOT level relative to Control as shown in table 2 below. However, there was a dose-dependent significant (P<0.05) increase in the SGOT level when the two extract groups were compared. There was a significant (P<0.05) increase in the SGPT level and a significant (P<0.05) decrease in ALP and creatinine levels when the HS groups were compared with Control with the high dose HS being more significant (P<0.05) when compared with the low dose HS group. The low dose HS significantly (P<0.05) decreased while the high dose HS significantly (P<0.05) increased the urea level when compared with the Control. There also appeared to be a dose-dependent effect as the effect of the high dose on urea level was significantly (P<0.05) higher than that of the low dose HS.

Table 2: Effect of consumption of aqueous extract of *Hibiscus sabdariffa* L. during pregnancy on some biochemical parameters

	iu/L			mg/dL	
	SGOT	**SGPT**	**ALP**	**Urea**	**Creatinine**
Control	97.0 ± 1.97	36.0 ± 1.15	117.7 ± 1.69	47.40±0.30	2.09±0.02
0.6 g/100 ml	92.0 ± 2.21	44.0 ± 1.86*	113.93 ± 1.07*	46.47±0.37*	1.41±0.02*
1.8 g/100 ml	101.0 ± 2.69[a]	57.0 ± 1.44*[a]	94.8 ± 1.05*[a]	57.6±0.26*[a]	1.00±0.01*[a]

* = P<0.05 compared with Control; a = P<0.05 compared with 0.6 g/100 ml. n = 6 rats per group.

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Length of Gestation, Litter Size and Birth Weight

There was no significant (P>0.05) difference in the length of gestation among the dams in the various groups as shown in the table 3 below. The litter sizes and the litter birth weights of the HS dams were significantly (P<0.05) lower and higher respectively than that of the control dams.

Table 3: Effect of consumption of aqueous extract of *Hibiscus sabdariffa* L. during pregnancy on length of gestation, litter size and birth weight

Groups	Length of gestation (days)	Litter size (n)	Litter birth weight (g)
Control	21.50±1.61	8.67±0.67	5.61±0.14
0.6g/100ml	22.15±1.07	6.50±0.76*	6.00±0.14*
1.8g/100ml	21.75±0.75	4.67±0.88*	6.11±0.12*

N = 6 rats per group, * = P<0.05 compared with Control.

Discussion

It is well established that the ability of a hepatoprotective agent to decrease the severity of the injury induced by hepatotoxic agents is the hallmark of its protective effect. Although, serum plasma level is not a direct measure of hepatic injury (Ramakrishna et al., 2011), elevated levels of SGOT and SGPT are widely used as indicators of hepatocellular injury (Yue et al., 2006) since the lowering of their levels are definite indicators of hepatoprotection (Ramakrishna et al., 2011). SGOT and SGPT are both associated with the liver parenchyma cells. SGOT is not a specific marker for hepatotoxicity since it is also found in red blood cells, brain, kidneys, cardiac and skeletal muscles whereas SGPT is more specific as it is localized mainly to the liver (Wurochekke et al., 2008).

Even though it has been reported that HS is hepatoprotective against induced hepatotoxity, some degree of hepatotoxicity, characterized by significantly elevated SGPT, was observed in the present study. This may suggest that aqueous extract of HS, at the doses tested may have some hepatotoxic effect, in terms of increasing the plasma level of SGPT, on the intact liver of pregnant rats. The liver uses SGOT and SGPT to metabolize amino acids and to make proteins. When the liver cells are damaged or the permeability of the hepatocyte membrane is altered these enzymes leak out into the bloodstream (Ramakrishna et al., 2011; Jasmine and Daisy, 2007) where they engage in gluconeogenic activities (Scott et al., 1984; Hossain et al., 2011). This mobilizes glucose into the bloodstream, thereby, increasing the blood glucose level (Asaduzzaman et al., 2010).

ALP is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in the plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. The observed significant reduction in the ALP level in the present study may therefore suggest a normal functioning of the hepatic ducts architecture.

Urea is the main end product of protein catabolism whose concentration varies directly with protein intake and inversely with the rate of excretion (Wurochekke et al., 2008). Nephrotoxicity leads to urea retention while hepatotoxicity leads to decrease in urea concentration (Ranjna, 1999). Creatinine is a waste product formed in muscle by creatine metabolism. Creatine is synthesized in the liver and transported through the circulation to the muscle and brain (Taylor, 1989). Its retention in the blood is evidence of kidney impairment (Wurochekke et al., 2008).

In the present study, there were dose-dependent increases and decreases in the urea and creatinine levels respectively. This may suggest a normal functioning of these organs. Since some degree of hepatotoxicity was suggested following the elevated level of SGPT, the increased urea concentration in this study may therefore be a result of the increased gluconeogenic activities of the transaminases SGOT and SGPT. It is suggested that the protein substrate may have been derived in whole or in part from the aqueous extract of HS consumed. This is so because phytochemical analysis of the extract has shown that protein is abundantly present (Adigun et al., 2006).

It has been reported that aqueous extract of HS at the doses tested significantly decreases fluid and food intake (Iyare et al., 2010a, b) and maternal malnutrition during pregnancy has been shown to result in low birth weight offspring (Iyare and Nwagha, 2009; Barker, 2000; Seckl, 1998; Philips et al., 1998; Lesage et al., 2001). In the present study, however, there was increased birth weight in the HS groups.

When decreased food consumption occurs early in pregnancy, before the development of the placenta (as occurred in the HS groups in the present study since HS consumption started on day 1 of pregnancy), it causes the development of a placenta with an increased efficiency to transfer nutrients to the developing foetus (Fowden and Forhead, 2004; Pond et al., 1991; Woodall et al., 1996; Osgerby et al., 2002; Jensen et al., 2002; Ain et al., 2005; Fowden et al., 2006). This, coupled with the increased gluconeogenic activities of the transaminases SGOT and SGPT, implies increased nutrient delivery and growth of the foetus. This may have resulted in the increased litter birth weight observed for the HS groups in the present study.

Conclusion

From the results of the present study, it can be concluded that consumption of aqueous extract of HS during pregnancy increases the litter birth weight possibly through the increased gluconeogenic activities of the elevated SGPT level that provides more glucose to the developing foetus possibly through a more efficient placenta.

Conflict of Interest Statement: We declare that we have no conflict of interest.

References

1. Adigun, M.O., Ogundipe, O.D., Anetor, J.I.and Odetunde, A.O. (2006). Dose-dependent changes in some haematological parameters during short-term administration of *Hibiscus sabdariffa* calyx aqueous extract (zobo) in Wistar albino rats. African Journal of Medicine and Medical Science, **35**: 73-77.
2. Ain, R., Canhan, L.N. and Soares, M.J. (2005). Dexamethasone-induced intrauterine growth retardation impacts the placental prolactin family, insulin-like growth factor-11 and Akt signaling pathway. Journal of Endocrinology, **185**; 253-263.
3. Ali, B.H., Mousa, H.M. and El-Mougy, S. (2003). The effect of a water extract and anthocyanins of *Hibiscus sabdariffa L*. on paracetamol-induced hepatotoxicity in rats. Phytotherapy Research **17**: 56–59.
4. American Physiological Society. (2002). Guiding principles for research involving animals and human beings. American Journal of Physiology: Regulatory Integrative and Complementary Physiology, **283**:R281-R283.
5. Amin, A. and Hamza, A.A. (2005). Hepatoprotective effects of Hibiscus, Rosmarinus and Salvia on azathioprine-induced toxicity in rats. Life Science, **77**(3):266-78. Epub 2005 Feb 17.
6. Appel, S.D. (2003). Red sorrel, *Hibiscus sabdariffa*-The other "cranberry". Plants and Gardens News, **18**:1- 2.
7. Asaduzzaman, M., Akhtar, M.A., Islam, M.A., Khan, M.R.I., Anisuzzaman, A.S.M. and Ahmed, M. (2010). Evaluation of antidiabetic, antihyperlipidaemic and hepatoprotective effects of *Allium sativum* (Linn.) in alloxan induced diabetic rats. Bangladesh Pharmaceutical Journal, **13** (1): 28-33.
8. Asagba, S.O., Adaikpoh, M.A, Kadiri, H.and Obi, F.O. (2007). Influence of aqueous extract of *Hibiscus sabdariffa* L. petal on cadmium toxicity in rats. Biological Trace Element Research, **115** (1):47-57.
9. Barker, D.J. (2000). *In utero* programming of cardiovascular diseases. Theriogenology, **53**: 555-574.
10. Clydesdale, F.M.C. (1979). Roselle (H.S.L.) anthocyanins as colourants for beverages and gelatin deserts. Journal of Food Protection, **42**: 204-267.
11. Dafallah, A.A. and Al-Mustafa, Z. (1996). Investigation of the anti-inflammatory action of *Acacia nilotica* and *Hibiscus sabdariffa*. American Journal of Chinese Medicine, **24**: 263-269.
12. Duke, C.T. and Francis, F.J. (1973). Anthocyanins of rosella (*Hibiscus sabdariffa*, L.). Journal of Food Science, **38**: 810–812.
13. Fowden, A.L. and Forhead, A.J. (2004). Endocrine mechanisms of intrauterine programming. Reproduction, 127: 515-526.
14. Fowden, A.L., Ward, J.W., Wooding, F.P.B., Forhead A.J., and Constanci, M. (2006). Programming placental nutrient transport capacity. Journal of Physiology, **572**: 5-15.
15. Fuleki, T., and Francis, F.J. (1968). Determination of total anthocyanin and degradation index. Journal of Food Science, **33**: 78-82.
16. Hossain, M.S., Ahmed, M . and Islam, A . (2011). Hypolipidemic and hepatoprotective effects of different fractions of methanolic extract of *Mormodica charantia* (Linn.) in alloxan induced diabetic rats. International Journal of Pharmaceutical Sciences and Research, **2** (3): 601-607.
17. Iyare, E.E., Adegoke, O.A. and Nwagha, U.I. (2010a). Mechanism of the decreased food consumption and weight gain in rats following consumption of aqueous extract of the calyx of *Hibiscus sabdariffa* during pregnancy. Asia Pacific Journal of Tropical Medicine, **3**(3): 185-188.
18. Iyare, E.E., Adegoke, O.A. and Nwagha, U.I. (2010b). Mechanism of delayed puberty in rats whose mothers consumed *Hibiscus sabdariffa* during lactation. Pharmaceutical Biology, **48** (10): 1170-1176.
19. Iyare, E.E. and Nwagha, U.I. (2009). Delayed puberty onset in rats that consumed aqueous extract of *Hibiscus sabdariffa* during the juvenile-pubertal period. Pakistan Journal of Biological Sciences, **12** (23): 1505-1510.
20. Jasmine, R. and Daisy, P. (2007). Hypoglycemic and hepatoprotective activity of *Eugenia jambolana* in streptozotocin-diabetic rats. International Journal of Biological Chemistry, **1**: 117-121.
21. Jensen, E.C., Gallaher, B.W., Breier, B.H. and Harding, J.E. (2002). The effect of a chronic maternal cortisol infusion on the late-gestation fetal sheep. Journal of Endocrinology, **174**, 27–36.
22. Lesage, J., Blondeau, B., Grino, M. and Dupouy, J.P. (2001). Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids, intrauterine growth retardation and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. Endocrinology, 142: 1692-1702.
23. Liu, C.L., Wang, J.M., Chu, C.Y., Cheng, M.T. and Tseng, T.H. (2002). In vivo protective effect of protocatechuic acid on tert-butyl hydroperoxide-induced rat hepatotoxicity. Food and Chemical Toxicology, **40** (5):635-41.
24. Mallie, J.P. and Boudzoumou, P. (1996). Functional renal maturation in rats neonates after prenatal exposure to furosemide. Pediatric Nephrology, **10**: 458-460.
25. Morton, J.F. (1987). Roselle. In: Fruits of warm climate. Juliaf, Morton and Miami, Florida.281-286.
26. Olaleye, M.T. and Rocha, B.T. (2008). Acetaminophen-induced liver damage in mice: effects of some medicinal plants on the oxidative defense system. Experimental and Toxicologic Pathology, **59** (5):319-27. Epub 2007 Dec 3.
27. Oliver, B. (1960). Medicinal plants in Nigeria. Nigerian College of arts, Science and Technology, Ibadan, 16-42.
28. Osgerby, J.C., Wathes, D.C., Howard, D.and Gadd, T.S. (2002). The effect of maternal undernutrition on ovine fetal growth. Journal of Endocrinology, **173**: 131-141.
29. Perry, L.M. (1980). Medicinal plants of east and southeast Asia. MIT press, Cambridge.
30. Philips, D.I., Barker, D.J., Fall, C.H., Seckl, J.R., Whorwood, C.B., Wood P.J. and Walker, B.R. (1998). Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome? Journal of Clinical Endocrinology and Metabolism, **83**: 757-760.
31. Pond, W.G., Maurer, R.R. and Klingt, J. (1991). Fetal organ response to maternal protein deprivation during pregnancy in swine. Journal of Nutrition, 121; 504-504.
32. Raji, Y., Morakinyo, A.O., Oloyo, A.K.. Akinsomisoye, O.S., Kunle-Alabi, O.T., Esegbue P.R.C. and Awobajo, F.O. (2005). Impact of chloroform extract of *Carica papaya* seeds on estrous circle and fertility in female albino rats. Journal of Medical Science, **5**: 337-43.

http://dx.doi.org/10.4314/ajtcam.v12i5.12

33. Ramakrishna, S., Geetha, K.M., Bhaskar gopal, P.V.V.S., Ranjit kumar, P., Charan Madav P. and Umachandar L. (2011). Effect of *Mallotus Philippensis Muell.-Arg* leaves against hepatotoxicity of Carbon tetrachloride in rats. International Journal of Pharmaceutical Sciences and Research, 2(2): 74-83.
34. Ranjna, C. (1999). Practical Clinical Biochemistry Methods and Interpretation. 2nd Edn, 117.
35. Scott, F.W., Trick, K.D., Lee, L.P., Hynie, I., Heick, H.M. and Nera, E.A. (1984). Serum enzymes in the BB rat before and after onset of the overt diabetic syndrome. Clinical Biochemistry, **17** (4): 270-275.
36. Seckl, J.R. (1998). Physiologic programming of the fetus. Clinical Perinatology, **25**: 939-962.
37. Taylor, E.H. (1989). Clinical Chemistry. New York: John Wiley and Sons, **4**, 58-62.
38. Wang, C.J., Wang, J.M., Lin, W.L., Chu, C.Y., Chou, F.P. and Tseng, T.H. (2000). Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxideinduced hepatic toxicity in rats. Food and Chemical Toxicology, 38: 411–416.
39. Woodall, S.M., Breier, B.H., Johnston, B.M., and Gluckman, P.D. (1996). A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotrophic axis and postnatal growth. Journal of Endocrinology, **150**: 231-242.
40. Wurochekke, A.U., Anthony, A.E. and Obidah, W. (2008). Biochemical effects on the liver and kidney of rats administered aqueous stem bark extract of *Xemenia americana.* African Journal of Biotechnology, **7** (16), 2777-2780.
41. Yue, M., Yu, C.H., Ren, K., Chen W. and Li, Y. (2006). Transient elevation of hepatic enzymes in volunteers after intake of alcohol. Hepatobiliary and Pancreatic Diseases International, **5** (1): 52-55.