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Effect of "Foléré" Juice (Calyx of *Hibiscus sabdariffa* Lin) on Some Biochemical Parameters in Humans

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Abstract: This study aimed to evaluate the effect of the consumption of "Foléré" juice extracted from the dried calyx of *Hibiscus sabdariffa* Lin on some biochemical parameters in humans. A standardized extraction procedure of the juice was set up using a survey carried out on 10 "foléré" juice sellers in Douala town. An enrolment of 22 apparently healthy male volunteers was done and these were observed before and throughout the study period. Each subject consumed 1 liter of juice per day (half in the morning and half in the evening). Glucose, haemoglobin, serum iron, total cholesterol, triglycerides, High density lipoprotein cholesterol, low density lipoprotein cholesterol, aspartate aminotransferase, alanine aminotransferase and C-reactive protein levels were all determined at days 0, 4 and 9. Results shows that "Foléré" juice significantly decreases the serum level of serum iron, total cholesterol and high density lipoprotein ($p<0.05$) and increases significantly triglycerides and haemoglobin levels ($p<0.05$). Also, no differences in blood glucose levels were obtained during the study period. Furthermore, a non significant decrease in transaminases levels was also observed. These findings suggest that "foléré" juice can safely be used in the prevention and management of anaemia and cardiovascular diseases and may also have some level of hepatoprotective effects as far as transaminases are concerned.

Key words: *Hibiscus sabdariffa* Lin, humans, haemoglobin, cholesterol, lipoproteins, transaminases

INTRODUCTION

In recent decades, nutritional science has clearly demonstrated the importance of nutrition for health and general well being. Populations have to consume enough foods with adequate nutritional quality and safety to meet all micro- and macronutrient requirements (Kraisid and Pattanee, 2009). Consumers are looking for variety in their diets and are aware of the health benefits of food rich in micronutrients. Regular consumption of plant foods are associated with numerous health benefits rooted in their various physiological effects as a result of their phytochemical and nutritional constituents (Hunter and Fletcher, 2002). Cell culture systems and animal models have provided wealth of information on the effects of nutrients, from vegetables and fruits and on the mechanism by which diets rich in vegetables and fruits are particularly important in promoting health such as reducing risk of cardiovascular diseases (Visioli *et al*., 2000; Gupta and Prakash, 2009).

Hibiscus sabdariffa Lin (Hs) from the malvaceae family, found in many tropical areas may have a good nutritional potential (Morton *et al*., 2000). Its hypocholesterolemic, antihypertensive, antioxidant, cardio protective, hepatoprotective effects have been investigated in mice, rabbits and rats (Chen *et al*., 2004; Carvajal-Zarrabal *et al*., 2005; Hirunpanich *et al*., 2006; Ologundudu *et al*., 2009). However, little studies have been done in humans. The "foléré" juice made up from the dried calyces of Hs recently became a popular soft drink in Cameroon. However its biological as well as pharmacological effects are still poorly understood even though for common peoples in Cameroon, it may have some positive effects on the blood.

The objective of this study was to investigate the effect of "foléré" juice on some physiological parameters such as haemoglobin, serum iron, lipids profile and transaminases, so as to contribute in a better understanding of the biological properties of this rapidly increasing popular soft drink.

MATERIALS AND METHODS

Juice preparation: The "foléré" juice was prepared using the results of the survey carried out on 10 "foléré"

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Table 1: Anthropometric characteristics of subject throughout the study period (n = 22)

	Days			
	D0	D4	D9	Normal range
Normal BMI (kg.m^{-2})	22.98±0.35* (n = 16)	22.69±0.42 (n = 15)	22.77±0.38 (n = 15)	18.5-24.9
High BMI (kg.m^{-2})	27.93±0.62 (n = 6)	27.43±0.60 (n = 7)	27.61±0.63 (n = 7)	25-29.9
SP (x10 mmHg)	10.82±0.23	11.05±0.20	11.14±0.16	<14
DP (x10 mmHg)	6.23±0.18	6.50±0.17	6.09±0.17	<9

*Means±SD. SP: Systolic Pressure, DP: Diastolic Pressure, n: number of subject, D0: Day 0, D4: Day 4, D9: Day 9, BMI: Body Mass Index

sellers in Douala town. For preparing 20 litres of juice, 1.5 kg of pineapple peel were boiled in a pot with 12 litres of water for 30 min, followed by the addition of 430g of previously washed dried calyx of Hs. The mixture was allowed for a further 10 min boiling using a gas cooker fire. After cooling, the mixture was filtrated to obtain the red filtrate. Concomitantly, 2 kg of sugar powder was dissolved in 8 litres of water and boiled for 5 min. That solution was added to the red filtrate obtained as described above and homogenized to obtain the "Foléré" juice. The juice was then kept at 4°C using polyethylene bottles of 1 litre.

Study subjects: Twenty two male subjects were recruited for the study (aged 19 to 39 years with a mean of 25.95±5.26 years). Table 1 describe the anthropometric characteristics of subject during the period of the study. None of the subject had reported of taking "foléré" juice one month prior to the study and they were advised not to consume "foléré" juice from another source. The consumption of the juice covered a 8 days period during which each subject was to take 1 litre a day (500 ml in the morning and 500 ml in the afternoon). No change was to happen in the normal food diet routine of the subjects, only that "foléré" was to be added as a supplement.

Collection and handling of blood samples: The collection of fasting blood sample were performed on days 0, 4 and 9 between 6.00 and 8.00 AM in a private health centre at Douala known under the name PRO-SA-COM (Promotion pour la Santé Communautaire). Blood samples were collected in tubes with and without EDTA as anticoagulant. Serum obtained after centrifugation of the blood collected in the tube without anticoagulant was divided in two set: the first was used the same day for glucose determination and the second was stored at -80°C for further analyses. Blood collected in the tubes with anticoagulant was used the same day to investigate haemoglobin level.

Biochemical analyses: Haemoglobin (Hb) and Serum iron (SFe) were measured spectrophotometrically using standard commercial kits (QUIMICA CLINICA APLICADA [Amposta, Spain] and RANDOX [Antrim, UK] respectively) while Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL) and glucose were measured using SGM Italia (Roma, Italia) standard kit. The concentration of Low Density Lipoprotein Cholesterol (LDL) was determined using the Friedewald formula (Friedewald *et al*., 1972). Alanine aminotransferase (ALT) and C-reactive protein (CRP) were measured using BIOREX kit (Antrim, UK), while Aspartate Aminotransferase (AST) was measured using FLUITEST GOT ASAT kit (Lichtenfels, Germany).

Statistical analyses: Results were reported as means ± SD and statistical analyses were done using Graph pad prism version 5.00 software. The following statistical tests were performed: one way ANOVA, Kruskall-Wallis and Bonferroni. A P value of less than 0.05 was considered significant.

RESULTS

Effect of "foléré" juice on haemoglobin and serum iron: "Foléré" Juice had a remarkable effect on haemoglobin (Hg) and Serum iron (SFe) as the mean values of these parameters (Fig. 1) were respectively increasing and decreasing during the study. For haemoglobin, we obtained mean values of 13.96±1.07, 14.35±1.08, 17.51±1.29 g/dl for D0, D4 and D9 respectively ($p = 0.021$). As for serum iron, the mean values were 86.17±9.26, 52.27±10.4, 59.73±9.72 µg/dl for D0, D4 and D9 respectively ($p = 0.042$).

Effect of "foléré" juice on the lipid profile: A complete lipid profile including Total Cholesterol (TC), Triglycerides (TG), HDL was investigated and LDL as well as the LDL/HDL ratio was calculated afterward. The average serum TC level significantly decreases from 1.85±0.09 g/l on D0 to 1.73±0.08 g/l on D9 ($p = 0.03$). HDL follows the same trends as we obtained mean value of 0.66±0.05 g/l on D0 and 0.53±0.04 g/l on D9. However, means triglycerides levels significantly increase from 0.71±0.07 g/l on D0 to 1.23±0.08 g/l on D9 ($p<0.0001$). LDL and the LDL/HDL ratio were not significantly different during the course of the study (Fig. 2).

Effect of "foléré" juice on blood transaminases and glucose: The effect of "foléré" juice on liver functions was assessed by investigating blood transaminases: Aspartate Aminotransferase (AST) and Alanine Amino Transferase (ALT). The average AST and ALT levels

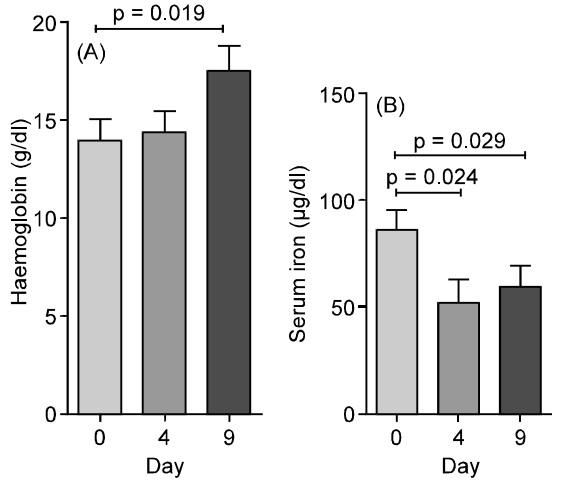

Fig. 1: Effect of "Foléré" juice on haemoglobin and serum iron. (A) haemoglobin; (B) serum iron

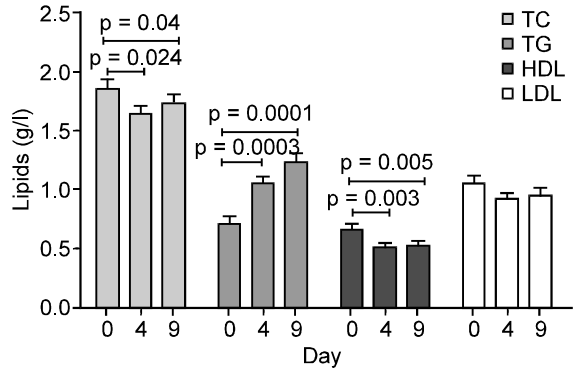

Fig. 2: Effect of "Foléré" juice on lipid profile. TC: Total Cholesterol; TG: Triglycerides; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein

decrease from D0 to D9 (as seen on Fig. 3) but was not significantly different after statistical analyses. The means of AST level on D0, D4 and D9 were respectively 35.91±2.16, 32.23±2.21 and 33.27±1.38 UI/l ($p = 0.23$), while the means of ALT level were respectively 31.36±2.20, 26.86±2.45, 29.32±1.67 UI/l ($p = 0.21$).

There were no significant differences (all p-values >0.05) in the participant subjects blood glucose levels throughout the study period. The mean glycaemia levels obtained on D0, D4 and D9 were respectively 0.87±0.03, 0.87±0.33 and 0.91±0.03 g/l (Fig. 4). CRP results of the subjects during the study indicate that some of them had minor infections as detected by this method.

DISCUSSION

Iron is a micronutrient necessary for respiratory gas transport via haemoglobin and it enters in the constitution of enzymes such as catalases, peroxydases

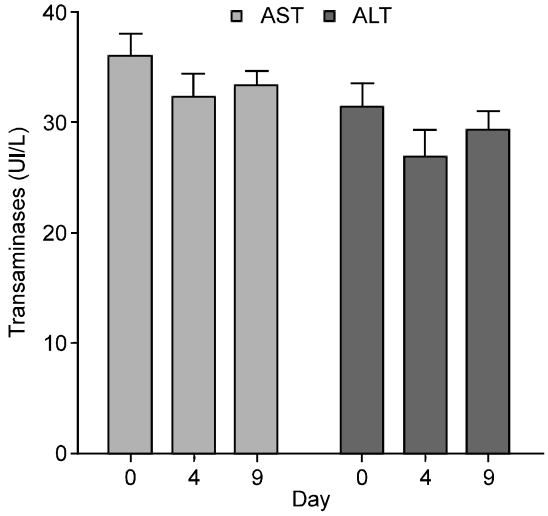

Fig. 3: Effect of "Foléré" Juice on blood transaminases. AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase

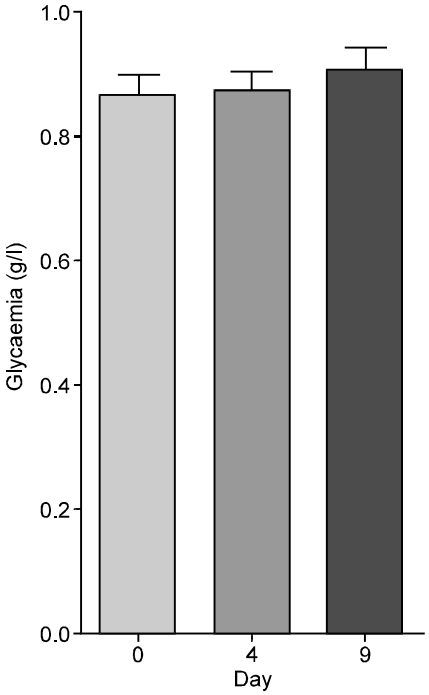

Fig. 4: Effect of "Foléré" juice on blood glucose

and cytochrome (Hercberg and Galan, 1991). Since Hb synthesis requires iron, this could explain the decrease of SFe throughout the study period. These results are similar to those of Ologundudu *et al*. (2009) who have studied the effect of anthocyanins extracted from the dried calyces of Hs on hematotoxicity induced by 2,4 dinitrophenylhydrazine in rabbit during 28 days. Also, the observed decrease of SFe level can be explained by the

quality of food consumed by the subjects and their physiological status as these parameters affect SFe status in human (Olsen and Nawiri, 2000). Furthermore, the result of CRP shows the presence of infections in some subjects, this may influence SFe variation during the study. The CRP is a marker of the presence of inflammation in an individual. When an individual is attacked by an endogen or hexogen causes, there is an increasing of inflammation protein levels proportionally to the intensity of the aggression.

The increasing of haemoglobin level may be due to the presence of anthocyanins in the calyx of Hs as it is known to induce the renal secretion of erythropoietin, an erythropoiesis hormone (Heda and Bhatia, 1986; Kaur and Kapoor, 2005). The function of haemoglobin is to transport respiratory gases between the lung and tissues. Therefore, as haemoglobin level increases in blood, the more will be the organism capacity to transport oxygen and to increase it vital functions (Lang, 1999). The haemoglobin concentration in this study has significantly increased; therefore we think that "foléré" juice could be used in the prevention of anaemia and could potentially increase vital capacity of people. Even though a decrease in SFe has been noticed with "foléré" consumption, this may not constitute a problem because the level remains in the normal range (59-158 µg/dl) recommended for humans.

Total cholesterol level decrease significantly during the study and these results are similar to those of Tzu-Li *et al.* (2007) who found that treatment with *Hibiscus sabdariffa* extract capsules reduce the mean serum total cholesterol level in their patients. We also found that "foléré" juice increase TG and decrease HDL levels significantly in human. In contrast, the above mentioned observations were different from the study of Promphorn *et al.* (2006) who found that Hs aqueous extract at doses of 250 and 1000 mg/kg/day given to male rats for 4 weeks could have no significant effect on lipids metabolism. These differences may be due to the difference in the duration of study (as our follow up period was only 8 days). Also, we worked on humans and not on rats.

Cardiovascular diseases can be influence by modifiable factors such as hyperlipidemia, tabagism, alcoholism and non modifiable factors such as age, sex and heredity (Kariola and Dedoussis, 2007). Hyperlipidemia is a risk factor of coronarian pathologies. The elevation of TC and LDL is associated to an increase of coronaries risk (Sue, 1994). During this study, TC level have decreased significantly and LDL not significantly ($p<0.05$). Therefore, "Foléré" juice may be used in the prevention of coronarian diseases. Furthermore, the risk of coronaries diseases increases with high blood pressure and body mass index (Kariola and Dedoussis, 2007). During the study, the means values of Body Mass Index (BMI), systolic pressure and diastolic pressure were not significantly different. The means values of blood pressure were within the normal range values recommended for human. But some subjects were overweight as far as BMI is concerned.

The decrease of some liver functions enzymes suggests some level of hepatoprotective effect in human as far as *Hibiscus sabdariffa* extract ("foléré" juice) is concerned, even though the difference was not significant after statistical analysis. The increase of AST and ALT levels is a sign of the deterioration of hepatocytes membrane integrity (Mahon *et al.*, 1990). In this study, AST and ALT have lowered and we suggest an influence of "foléré" juice consumption; consequently the "foléré" juice could have some hepatoprotective effect in humans.

With the utilization of sugar in "foléré" juice preparation, one could worried of the hyperglycaemia property of that juice. However, no statistical differences were obtained throughout the study period as far as the glycaemia levels of study subjects are concerned. Furthermore, these values were within the normal ranges, showing the safety of "foléré" juice consumption for study subjects

This study clearly demonstrates that "foléré" juice (Dried calyx of *Hibiscus sabdariffa* Lin) has some effects on several biochemical parameters in human. For instance, it usefulness in the prevention of anaemia and cardiovascular diseases as well as it harmlessness on liver (as far as transaminases are concerned) have been demonstrated. Further studies with an increased sample size and longer follow up, but also investigations involving other organs and parameters such as red blood cell count, red blood cell indices, white blood cell count are warranted for a more complete understanding.

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