Effect of the Oral Intake of Some Soft Drinks on the Fasting Blood Glucose Level and Lipid Profile of Albino Rats

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Abstract: The study was carried out to discover the effects of the oral intake of some soft drinks (Fanta, Sprite, Fayrouz, Coca-cola and Schweppes) on blood glucose level and lipid profile of albino rats. The rats were grouped into 6 each containing 4 rats, and were allowed to fast for 12hrs, their fasting blood glucose was taken to know their pre treatment fasting blood glucose level. Group 1 was administered with normal saline, group 2 was given Fanta, group 3 was given Sprite, group 4 was given Schweppes, group 5 was given Fayrouz and group 6 was given Cocacola. All the groups were administered with 3ml of the soft drink per 100g body weight twice a day respectively throughout the period of the research. The research lasted for 14 days. On the last day the rats were allowed to fast again for a period of 12hrs after which the fasting blood glucose level was determined; finally, the rats were sacrificed by cervical decapitation and the serum collected for the determination of lipid profile. There was statistical difference (p<0.05) in fasting blood glucose between the pre-treatment and post-treatment values. Group 2 (Fanta fed rats) and Group 6(Coca-cola fed rats) showed high level of glucose (p<0.05) compared to normal control, while the groups administered with Sprite, Schweppes and Fayrouz respectively showed no statistical difference in fasting blood glucose level when compared to the normal control. In the analysis of lipid profile, the group administered with Fanta exhibited high level of total cholesterol, low level of triglyceride and low density lipoprotein and normal level of high density lipoprotein. The group given Coca-cola showed low level of total cholesterol, triglyceride, high density lipoprotein and normal level of low density lipoprotein, while the group given Schweppes showed high level of total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein. The group given Fayrouz showed high level of Cholesterol, low density lipoprotein and low level of Triglyceride and high density lipoprotein. Lastly, the group given Sprite showed no statistical difference in all these parameters when compared to the control group. Therefore, it was concluded that among the various soft drinks used in the present study, it was only Sprite soft drink that appeared not to be implicated as a predisposing factor to any of the diseases considered.

Keywords: Soft drinks, Predisposing factor, Diabetes melitus, cardiovascular diseases, Lipid profile, Fasting blood glucose.

2.0 INTRODUCTION

Soft drinks also known as ready-to-drink beverages are sweetened water-based non alcoholic beverages mostly with balance acidity (Vartanion *et al.*, 2007). They are frequently flavored and colored and the principal component being water which is needed for hydration. Soft drinks are commonly consumed by both young and old people (Ebbling *et al.*, 2006; Malik *et al*, 2006).

A notable finding of Wolff and Dangsinger (2008), was that weight gained was more dramatic from soft

drinks compared with fruit punches and fruit juice. In addition intake of fruit juice was not associated with an increased risk of type-2 diabetes. This could be because of the low glycemic index (GI) of fruit juice, soluble fibre, or other constituents of fruit juice that could be beneficial, as the authors suggested (Caroline, 2004).

Previous study revealed that a woman with high intake of sugar-sweetened soft drinks tends to be less physically active; in addition, she has high total calories and low protein, alcohol, magnesium and



L. J Goje (Correspondence) Lazgoje@yahoo.com cereal fiber (Wolff and Dangsinger, 2008). Also, intake of total carbohydrate, sucrose and fructose as well as overall glycemic index was high in this woman. In essence this woman has dietary pattern and lifestyle that lead to increase risk of several disease states, including obesity, type -2 diabetes and cardiovascular disease (Gibson and Sigrid, 2008). Perhaps the take home message is that sugar sweetened beverages consumption can alert the primary care clinician to patient's unhealthy eating habit and lifestyle. Sugar sweetened beverages consumption as a marker of an unhealthy lifestyle has the potential of being a quick screening test for increase of obesity and type-2 diabetes, but it requires validation (Ludwig et al., 2001;Schulze, 2004;Dubois and Bankaaskait, 2005; Ashurst, 2009).

Because of the large amount of calories in sugar sweetened soft drinks and the relationship between the consumption of these drinks and weight gained, reducing sugar-sweetened beverage consumption may be the simple opportunity to curb the obesity epidemic (Kaben et al., 2002; Nielson and Popkin, 2004; Wolff and Dangsinger, 2008). Obesity is now a complex worldwide problem, resulting from a combination of genetic, behavioral, cultural and environmental influence that calls for not only behavioral changes at individual levels, but also changes in public policy, social, environmental, and cultural norms (Ripsin et al., 2009; Konstantinos, 2012). The world health organization (WHO) and the food and agriculture organization submitted a report in April, 2003 concluding that many death attributed to chronic diseases are due to obesity and outlined how million of people around the world can avoid chronic diseases through diet and exercise (Ripoll et al.,2011).

In the 1960s, for example, diabetes mellitus was said to be rare in the African continent with a prevalent rate of 0.5% and the prevalent rate, then, in south Africa and north Africa being the highest, but in 1992, Nigeria has a prevalent rate of 2.8% as discovered by the Nigerian National Expert Committee on non-communicable diseases with more in Urban compared prevalence to rural areas.(Santaguida et al., 2008). This is as a result of gradual westernization, leading to increase in the number of soft-drinks manufacturing companies. The traditional habit of giving water to a visitor is now replaced by soft drinks or alcohol, hence the need to assess their effects in raising the blood glucose concentration, and lipid profile being the major factors associated with diabetic mellitus and cardiovascular diseases. Diabetes mellitus is characterized by hyperglycemia together with the biochemical alteration of glucose and lipid peroxidation (Trocho et al., 1998; Lambert and Bingley, 2002; Rother, 2007). Lipid peroxidation, a free radical related process, is an uncontrolled, selfenhancing process causing disruption of membrane lipid and other cell components. Unlimited lipid peroxidation (LP) could be one of the main factors in the pathogenesis of diabetic complications (Stewart et al., 2007; Shoback, 2011). This pathology is often related to the release of free radicals and caused oxidative stress (Sadah et al., 2001). During re oxygenation hypoxanthine/ xanthine oxidase and arachidonic acid pathways are important sources of free oxygen radicals, which damage lipid membrane and lead to cytolysis and cell death (Domer, 1997). The accumulation of lipid in diabetes is mediated through a variety of derangement in metabolic and regulatory process especially insulin deficiency thereby rendering the diabetic patient more prone to hypertriglyceridemia hypercholesterolemia and (Jaiprakash et al., 1993). One of the major pathogenesis of lipid metabolism, disturbances in diabetes, is the increase mobilization of fatty acid from adipose tissue and secondary elevation of free fatty acid level in blood (Vijan, 2010). Lipid hypercholesterolemia, abnormalities such as hypertriglyceridemia, hyperphospholipidemia and fatty acid distribution changes are common in diabetic patients (Konstantinos, 2012). Consumption of soft drinks in Nigeria is becoming high, and this soft drinks are reported to contained high calories which tend to increase the risk of obesity, type-2 diabetes and cardiovascular diseases (Selvin et al., 2010). Therefore, there is need to study the effects of these soft drinks through the assessment of these parameters as indices that point to predisposure to diabetes mellitus and cardiovascular diseases in order to reduce the risk of obesity, type-2 diabetes and cardiovascular diseases.

AIM AND OBJECTIVES OF THE STUDY

The aim and objectives of this research is to study the effects of some soft drinks (Fanta, Coca-cola, Sprite, Fayrouz and Schweppes) on blood glucose level and lipid profile of albino rats, as markers of diabetes mellitus and cardiovascular diseases.

3.0 MATERIALS AND METHODS 3.1 Experimental animals

Twenty four (24) Albino Winstar rats weighing between (220-250g) were used for the present study and were purchased from Federal College of Veterinary and Medical Laboratory Technology (FCVMLT) Vom, Jos Plateau State, Nigeria. The animals were acclimatized for two weeks and they were maintained on water and animal feed (Vital feed).

The 24 Winstar albino rats used in this research were divided into six (6) groups containing four (4) rats each as follows:

Group 1 (Control). The rats in this group were fed with 3ml of normal saline, intragestrically per 100g body weight.

Group 2 (Fanta). The rats in this group were fed intragestrically with Fanta at a concentration of 3ml/100g body weight.

Group 3 (Sprite). The rats in this group were given Sprite intragestrically at a concentration of 3ml/100g body weight.

Group 4 (Schweppes). The rats in this group were fed intragestrically with Schweppes at a concentration of 3ml/100g body weight.

Group 5 (Fayrouz). The rats in this group were administered with Fayrouz at a concentration of 3ml/100g body weight throughout the period of the research

Group 6 (Coca-cola). The rats in this group were given Coca cola intragestrically at a concentration of 3ml/100g body weight.

The soft drinks were administered twice a day, in the Morning and Evening for a period of fourteen (14) days.

3.2 Soft drinks

The soft drinks (Coca cola, Sprite, Fayrouz, Fanta and Schweppes) used in this study were obtained from Cool drinks Shopping Complex No.2 Gombe central market. They were opened just before the room temperature to remove excess gas before administration and were all administered intragestrically.

Finally, the rats were sacrificed by cervical decapitation; the blood samples were collected in plain containers and centrifuged at 3000rpm for 15 minutes at 37^{0} C degrees Celsius. The Serum was separated using Pasteur pipette for the determination of the lipid profile.

3.3 Determination of fasting blood glucose Principle

Glucose in the blood sample mixes with special chemicals on the test strip and a small electrical current is produced. This current is measured by the one touch ultra-meter and displayed as blood glucose result. The strength of this current change with the amount of glucose in the blood

Procedure

24 Winstar albino rats used in this study were allowed to fast for twelve (12) hours before the administration of soft drinks and the blood glucose level were taken by sterilizing their tails with 10% alcohol, and cutting the tails using scissors then allowing the blood to touch the test strip which was inserted into a calibrated glucose meter (One touch Glucometer, Acon Laboratory INC. San Diego, USA) .This gave direct reading after 5 seconds in mg/dL. The blood glucose level of the rats before the administration of soft drinks was measured in order to know the normal blood glucose of the rats in each group. After the administration of soft drinks on the last day, all the rats in the groups were fasted again for 12hrs and their fasting blood sugar was determined using glucose meter. This was done in order to check or observe the effect of soft drinks on blood glucose level when compared to their initial glucose level (before the administration).

3.4 Determination of lipid profile

Triglycerides

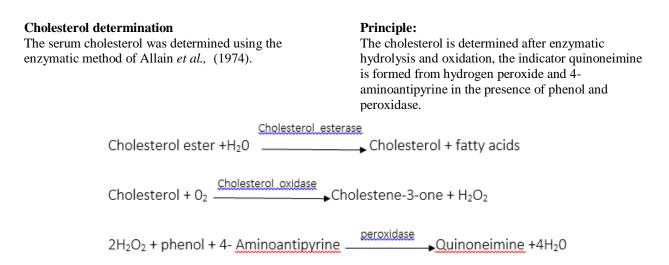
The enzymatic method for the determination of Triglyceride using GPO-PAP method.

Principle:

The triglycerides are hydrolysed by the enzyme lipase to produce glycerol and fatty acids. The enzyme glycerol kinase acts on glycerol in the presence of ATP to form glycerol-3- phosphate and ADP, the glycerol-3-phosphate is then oxidised by glycerol phosphate oxidase to dihydroxyacetone and hydrogen peroxide, oxygen is released from H_2O_2 in the presence of peroxidase, which oxidizes p-chlorophenol chromogen to form a coloured compound.

The absorbance of this coloured complex which is directly proportional to the amount of Triglyceride present in the sample is measure at 530nm using a Spectrophotometer.

Triglycerides +H₂O → Glycerol +Fatty acids
Glycerol +ATP Glycerol-3-phosphate +ATP
Glycerol-3-phosphate +0 ₂ $\xrightarrow{\text{Glycerol phosphate oxidase}}$ dihydroxyacetone +phosphate
+H ₂ O ₂
2H ₂ O ₂ +4-aminophenazone+ 4chlorophenolpquinoneimine+Hcl+4H ₂ O



Determination of High density lipoprotein (HDL)

The enzymatic method for determination of HDL was used.

Principle:

Low density lipoprotein (LDL) and chylomicron fraction are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL i.e. High Density Lipoprotein fraction which remains in the supernatant is determined.

Determination of low density lipoprotein (LDL).

The low density lipoprotein (LDL) concentration was calculated using the Friedewald formula. The formula is given below:-

(/)5-HDL

Statistical analysis.

The data was expressed as the Mean \pm SD of four different observations. Experimental results were statistically analyzed using the Student's t-test. Difference in Means at p<0.05 was considered significant.

4.0 RESULTS AND DISCUSSION. 4.1 Results

The tables 1.0 and 2.0 below showed the result of fasting blood glucose for both pre-treatment and post-treatment values in the experiment respectively. Table 3.0 showed the comparison between the pre-

treatment and post-treatment values of fasting blood glucose. There is significant difference (p<0.05) between the pre-treatment and post-treatment values with respect to the fasting blood glucose level in all the groups.

Fanta fed group, and Coca-cola fed group exhibited significantly high level of fasting glucose at (p<0.05) compared to the control, group fed with normal saline. Sprite, Schweppes and Fayrous fed groups showed no any significant difference (p<0.05) in fasting blood glucose level when compared with the control group.

Table 4.0 showed the result for lipid profile, in which Fanta fed group exhibited high level of total cholesterol, low level of triglyceride and low density lipoprotein and normal level of high density lipoprotein when compared to normal control. The group given Coca-cola showed low level of total cholesterol, triglyceride, high density lipoprotein and normal level of low density lipoprotein .Schweppes fed group exhibited high level of total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein while Fayrouz fed group showed high level of Cholesterol, low density lipoprotein and low level of Triglyceride and high density lipoprotein when compared to normal control.

Sprite fed group showed no statistical difference (p<0.05) in the level of total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein when compared to normal control.

Table 1.0 pre-treatment values for fasting blood glucose in mg/dL

• 1.0	pre deddin	ent fundeb i	or rasting t	Sioou grae	be ming an.		
	Groups	Control	Fanta	Sprite	Schweppes	Fayrouz	Coca-cola
	FBS(90.67±1	64.33±	66.33±	107.33±13.	124.33±	106.67±40.20
_	mg/dL)	2.50	4.61	9.50	31	67.95	

The values are expressed as Mean \pm SD for four different determinations (n=4). Values with different superscripts compared to normal control are statically different at (p<0.05)

1 able 2.01 ost a calificative rates for fasting block gracose in mg/all.	Table 2.0 Post-treatment values	s for fasting blo	od glucose in mg/dL.
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Groups	Control	Fanta	Sprite	Schweppes	Fayrouz	Coca-cola
FBS(mg/dL)	89.00±3.00	167.00±7.21*	88.33±25.38	77.33±19.34	72.33±15.37	169.00±31.43*

The values are expressed as Mean \pm SD for four different determinations (n=4). Values with different superscripts compared to normal control are statistically different at (p<0.05)

Table 3.0 Comparison between the pre-treatment and post treatment values of the fasting blood glucose in (mg/dL).

Groups	Control	Fanta	Sprite	Schweppes	Fayrouz	Coca-cola
Pre-treatment	90.67±12.50	64.33±4.61	66.33±9.50	107.00±13.31	124.33±67.95	106.67±40.20
Post treatment	89.00±3.00*	167.77±7.21*	88.33±25.38*	77.33±19.34*	72.33±15.37*	169.0±31.*

The values are expressed as Mean \pm SD for four different determinations (n=4).

The values with superscripts compared to pre-treatment groups are statistically different at (p<0.05).

Table 4.0 Lipid prot	file			
Groups	CHOL(mg/dL)	TG(mg/dL)	HDL(mg/dL)	LDL(mg/dL)
Control	70.00±1.00	77.00±1.00	27.00±2.00	39.00±1.00
Fanta	75.00±1.00*	72.33±2.00*	27.33±1.53	33.67±1.53*
Sprite	70.67±1.53	78.68±1.15	28.67±1.53	41.67±1.53
Schweppes	98.33±1.15*	80.00±1.00*	36.00±1.00*	50.33±1.53*
Fayrouz	83.67±1.53*	28.67±1.53*	21.67±0.58*	55.33±1.15*
Coca-cola	60.33±1.53*	59.00±1.00*	42.00±2.00*	40.00±1.00

The values are expressed as Mean \pm SD for four different determinations (n=4). Values with superscripts compared to normal control are statically different at (p<0.05).

4.2 Discussion.

In this study, the effects of some soft drinks on blood glucose level and lipid profile parameters are discovered. The Fanta fed group exhibited high fasting blood glucose level, total cholesterol, and decreased in low density lipoprotein, triglyceride and normal level of high density lipoprotein compared to the normal control. These were similar to the result of earlier works of Joseph *et al.*,(2000), that some soft drinks may exhibit rise in blood glucose and triacyl glycerol due to the high glycemic index and some factors such as caffeine, high fructose, aspartame and caramel contents.

Coca-cola appeared to show an increased level of fasting blood glucose, compared to the control group and also comparison between the pre-treatment and post treatment value of fasting blood glucose appeared to increase significantly. It also showed increase in HDL, decrease level of cholesterol, TG and normal level of LDL. Therefore, Coca cola is not implicated to be a predisposing factor for cardiovascular diseases arising from imbalances in the lipid profile parameters. The increase in the fasting blood glucose level of the Coca-cola group can be attributed to the presence of caramel which is a major ingredient in Coca-cola soft drinks and caramel colorant is incriminated as a cause of elevated liver enzymes and may be a potential cause of advance glycation end product which may promote insulin resistance (Vlassara et al., 2002). These findings clearly showed that while Coca-cola and Fanta may not predispose one to cardiovascular diseases as seen in the results of the parameters of lipid profile it cannot be safe for people with diabetic history and also diabetic patients to consume.

The group fed on Schweppes and Fayrouz showed normal levels of glucose compared to the normal control and it appeared to show a decrease in the level of fasting blood glucose when the pretreatment and post treatment values were compared. Both Fayrouz and Schweppes showed increase level of cholesterol and LDL, where as there is decrease level of TG and HDL in Fayrouz as opposed to an increase in these parameters in the case of Schweppes. These findings clearly showed that while Schweppes and Fayrouz may not cause an increase in blood glucose level, they have the potential tendencies of predisposing people who consume them habitually to type II diabetes, atherosclerosis, and/ or other cardiovascular diseases due to increased in cholesterol, LDL, decreased HDL, and increased TG which are the major implicating parameters.

In the case of Sprite all the parameters comprising fasting blood glucose and lipid profile appeared to be normal when compared to the normal control. This clearly suggests that the ingredients in sprite did not contained high concentration of sugar sweeteners, and/ or other flavoring agents that lead to an increase in glucose level and lipid profile when compared to other soft drinks. Therefore, sprite consumption cannot be said to be a predisposing factor for diabetes and other cardiovascular disease. Hence Sprite might be safe for consumption by the populace based on this research provided it yields similar findings in humans.

Conclusion.

In this study it was observed that some soft drinks consumed nowadays can predispose one to some diseases. Amongst the various soft drinks used in this study, it was observed that Fanta and Coca-cola soft drinks tend to increase the fasting blood glucose level, while Fayrouz and Schweppes soft drinks reduced the blood glucose level but lead to imbalances in lipid profile parameters. Sprite soft drinks did not show any negative effect on glucose level and lipid profile parameters, hence might be the Soft drink of choice for public consumption based on these findings.

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