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ASPARTAME INDUCED DYSLIPIDEMIA AND PLASMA HYPERVISCOSITY IN ALBINO WISTAR RATS

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ABSTRACT

Aspartame is a low-calorie sweetener that has been used for decades in place of sugar. However, there has been conflicting reports on the use and safety profile of aspartame associated with several diseases especially cancer and cardiovascular diseases. This study therefore aims to determine the effects of aspartame on some cardiovascular indices. Thirty seven (37) young Albino Wistar rats weighing between 110g-140g were used for this study. The rats were divided into five groups; control (5) received 5 ml of distilled water. Group 1 (n=8) received 5 ml of diet soda, group 2 (n=8) received 5 ml diluted (1:1) diet soda, Group 3 (n=8) received 5 ml aspartame 45 mg/kg b.w, Group 4 (n=8) received 5 ml of aspartame 22.5 mg/kg b.w. The various doses of aspartame and diet soda were administered orally daily for ten weeks. Weight of the rats were taken weekly, at the end of the experimental period, the rats were sacrificed and blood samples collected into plain sample bottles and tri sodium citrate bottles. Lipid profile (total cholesterol, HDL-C, LDL-C and triglyceride) were analyzed spectrophotometrically, fibrinogen and blood viscosity were also measured. Results showed that the aspartame and diet soda significantly increased total cholesterol, triglyceride, LDL-C, fibrinogen and plasma viscosity and significantly decreased HDL cholesterol. In conclusion, consumption of aspartame as a sweetener can impact negatively on cardiovascular haemodymic factors. Therefore, its intake should be discouraged.

Keyword: Aspartame, Lipid profile, Fibrinogen and plasma viscosity.

INTRODUCTION

Cardiovascular diseases (CVD) are regarded as a major cause of mortality globally. Approximately 17.9 million people died from CVDs in 2019, constituting 32% of all global deaths. Of these deaths, 85% were ascribed to heart attack and stroke (Mensah et al. 2019). Over three quarters of CVD deaths take place in low- and middle-income countries. Out of the 17 million premature deaths (under the age of 70) due to non-communicable diseases in 2019, 38% were as a result of CVDs, (WHO, 2019). A large percentage of cardiovascular diseases can be prevented by addressing behavioral risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol. Early detection of cardiovascular disease is very necessary so that management with counseling and medicines can begin. The effects of behavioral risk factors may show up in individuals as raised blood pressure, raised blood glucose, raised blood lipids, overweight and obesity,(WHO 2019).

Artificial sweeteners are a class of food additives that provide sweet taste without increasing caloric intake. Aspartame (ASP) is an example of an artificial sweetener that is widely used globally. According to Magnuson et al. (2016), ASP has been observed to be two

hundred times sweeter than sucrose and is used by people who want to reduce calorie intake or desire to maintain a normal weight thereby consuming low calorie sweeteners in place of sugar.

After oral administration, (ASP) is completely decomposed into three components: two amino acids (aspartic acid and phenylalanine) and methanol. These constituents are metabolized using the same pathways, as they are derived from food, such as meat, milk, fruits and vegetables (Butchko et al. 2002). ASP may be hydrolysed into its components in the gastrointestinal lumen, to be later absorbed into the circulation. At times, methanol is hydrolysed in the intestinal lumen with transportation of the aspartylphenylalanine dipeptide into mucosal cells, where it is metabolized to aspartate and phenylalanine and then absorbed into circulation. ASP may also be absorbed by intestinal mucosal cells, where it is hydrolysed to its component and then absorbed into the circulation without affecting the gut microbiota (Ruiz-Ojeda et al. 2019). Approximately 50% of the ASP molecule is phenylalanine (Phe), 40% is aspartic acid (aspartate, asp) and 10% is methanol (MeOH) (Prokic et al. 2014). ASP was first approved in 1981 first for limited use in solid foods; in 1996 its use was expanded by the U.S. Food and Drug Administration (FDA) as a general sweetener. The sweetener was approved for general use in the European Union in 1994 (EC Directive 1994). ASP is now present in more than 6,000 consumer packaged goods and in nearly 500 pharmaceutical products, including children's medicines (Aspartame Information Center 2005). In the United States, more than 70% of aspartame sales are attributed to soft drinks (American Dietetic Association 2004).

The acceptable daily intake (ADI) of ASP is currently 50 mg/kg body weight (b.w) in the United States and 40 mg/kg b.w in the European Union for both children and adults. Daily consumption of artificial sweeteners by women of childbearing age and by children has been estimated at 2.5–5.0 mg/kg b.w (Butchko et al. 2002). Despite the fact that ASP intake has been considered safe in daily acceptable amounts, experimental studies have reported otherwise. Most of these studies suggested adverse effects Davidson et al. (2011) and Feijó et al. (2014) and few suggested neutral or beneficial properties, (Peters et al. 2016 and Panget et al. 2020).

ASP within 15 -35mg/kg b.w significantly increased the levels of total cholesterol, triglycerides and low-density lipoprotein cholesterol in the rats (Adaramoye 2016 and Magda et al.2018). However, Satvinder et al. (2022) reported that long term (one year) intake of ASP did not result in any significant increase in lipid profile in rats. Due to discrepancies surrounding the safety in the consumption of ASP and the conflicting literature available, this study aimed to determine the effects of ASP as a sweetener and its use in diet soda on some cardiovascular parameters.

MATERIALS AND METHODS

Experimental animals: Thirty seven (37) young albino Wistar rats with an average weight of 110g – 140g were used for this study. The animals were purchased and cared for in the animal house of the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, and University of Benin, Benin City. They were kept in clean cages in a well-ventilated environment. They had access to clean water and sufficient feed ad libitum, in accordance with the guidelines of National Research Council Guide for the care of laboratory animals (NRC, 1996) and revised by (Mansour et al. 2017). The study was conducted for a period of twelve (12) weeks including a two-week acclimatization period.

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Experimental design: The rats were divided into five groups. Group 1 (n= 5) served as the control group, and was administered 5 ml of distilled water daily. Groups 2 and 3 (n= 8) were administered 5 ml of the undiluted diet soda and diluted diet soda (1:1 dilution) respectively. Groups 4 and 5 (n=8) were administered 5ml of high dose aspartame (45mg/kg b.w) and low dose aspartame (22.5mg/kg b.w) respectively. The high dose was based on the ADI while the low dose was half the high dose. All administration was orally given using a clean gavage. All rats were sacrificed after ten weeks of administration.

Collection of samples: At the end of the ten weeks the animals were observed for general physical characteristics, and were weighed. The rats were subjected to an overnight fast. By the next day, they were anesthetized by exposure to 5% chloroform, after which blood was collected via cardiac puncture into plain sample bottles and sodium citrate container. Serum was harvested from the plain bottles after clotting while citrated plasma was harvested from the sodium citrate containers.

Biochemical assays: Lipid profile consisting of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were assayed spectrophotometrically. Total serum cholesterol was analyzed using the method of Allain (1974) while HDL-C was analyzed using the method of Lopes (1977). Serum triglyceride was determined by the enzymatic method of Stein (1987). LDL was extrapolated from total cholesterol, triglyceride and HDL by the method of Friedwald (1972).

The plasma viscosity was expressed as the ratio of the flow-time for 1 ml of plasma sample to the same volume of distilled water (Reid and Ugwu, 1987). Fibrinogen was determined by method of Ingram (1952).

Statistical analysis: All data obtained during the experiment was analyzed using Graphpad Prism 8.0 software and one-way analysis of variance (ANOVA). Tukey's Honest Significant Difference post-hoc test was used to compare results among groups. Results were expressed as Mean ± SEM and *P* values of ($P \leq 0.05$) were considered statistically significant.

RESULTS

Figures I to IV compare mean values of lipid profile of the various groups with control, there was a significant increase ($P < 0.05$) in total cholesterol, triglyceride and LDL-C ($P < 0.001$) and a significant ($P < 0.05$) reduction in HDL-C in groups administered 45 mg/kg b.w ASP, while group 2 administered undiluted diet soda only showed significant increase in LDL-C ($P < 0.001$).

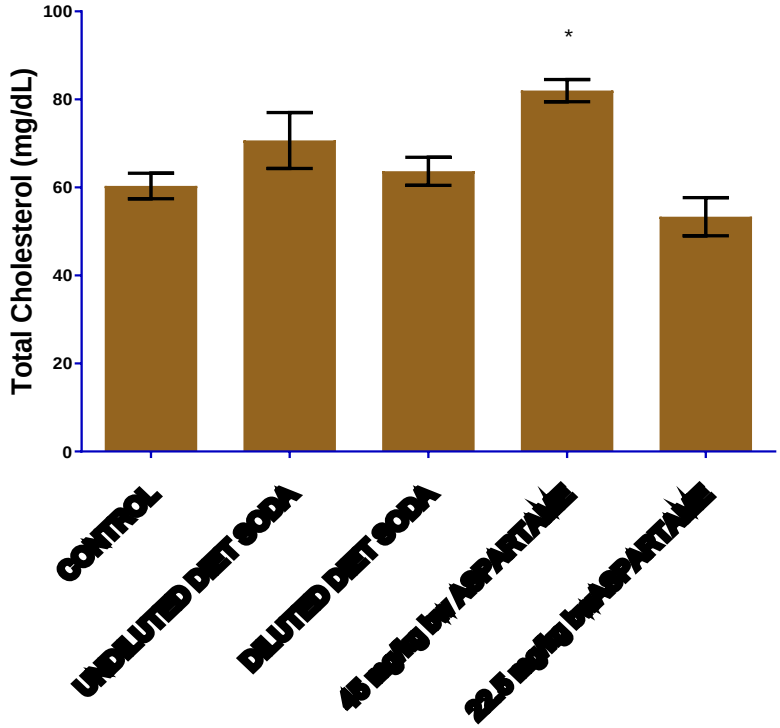

Figure I: showing the effect of diet soda and aspartame on total cholesterol TC Concentration

*- P<0.05, Results show there was a statistically significant increase in the 45 mg/kg b.w aspartame treated group compared with control ($P < 0.05$).

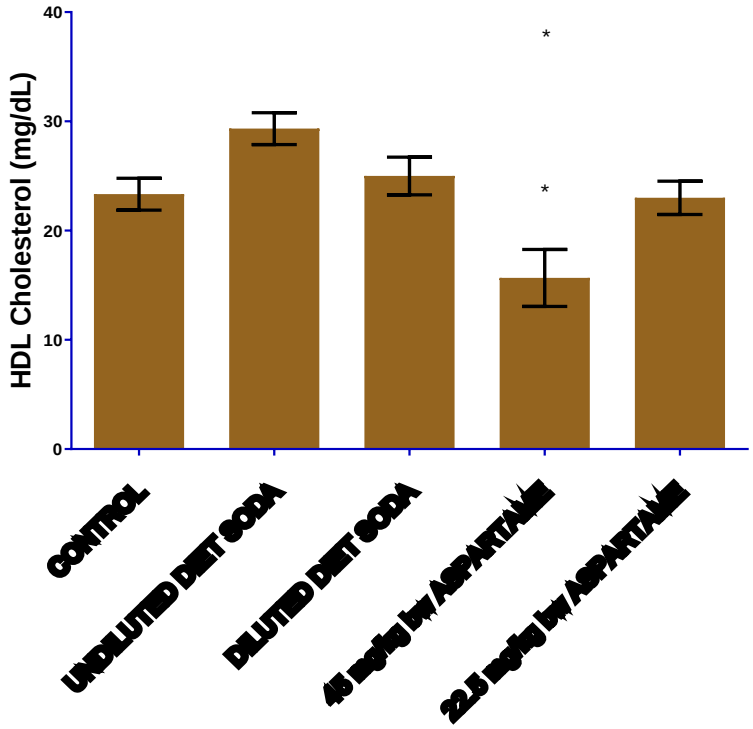

Figure II: showing the effect of diet soda and aspartame on HDL-c Concentration

*$P < 0.05$), results show there was a statistically significant decrease in the conc. aspartame treated group compared with control ($P < 0.05$).

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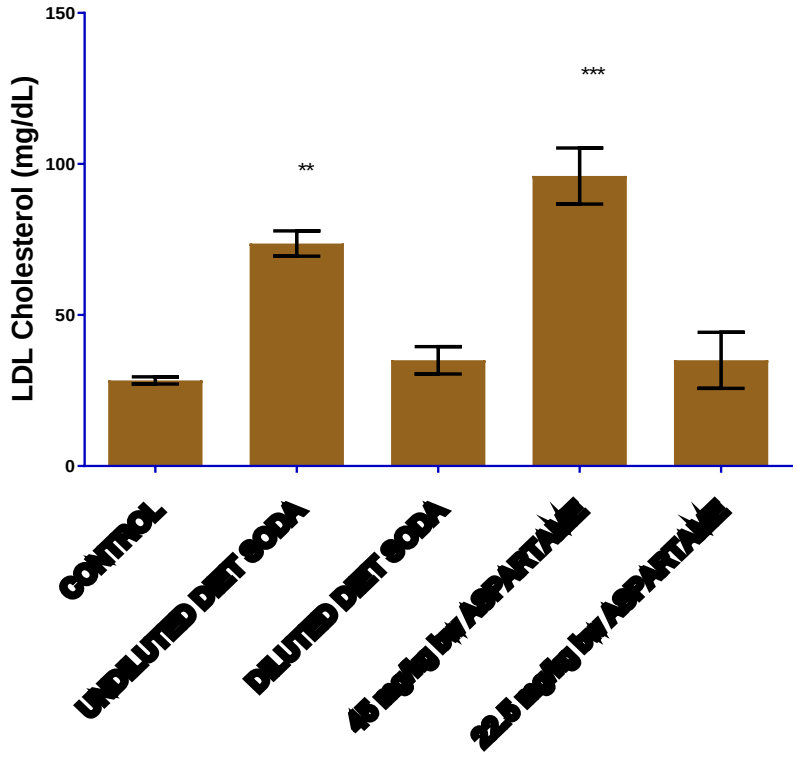

Figure III: showing the effect of diet soda and aspartame on LDL-C Concentration

** ($P < 0.01$), *** ($P < 0.001$), results show there was a statistically significant increase in the conc. Diet soda and conc. Aspartame (45 mg/kg b. w) treated group compared with control.

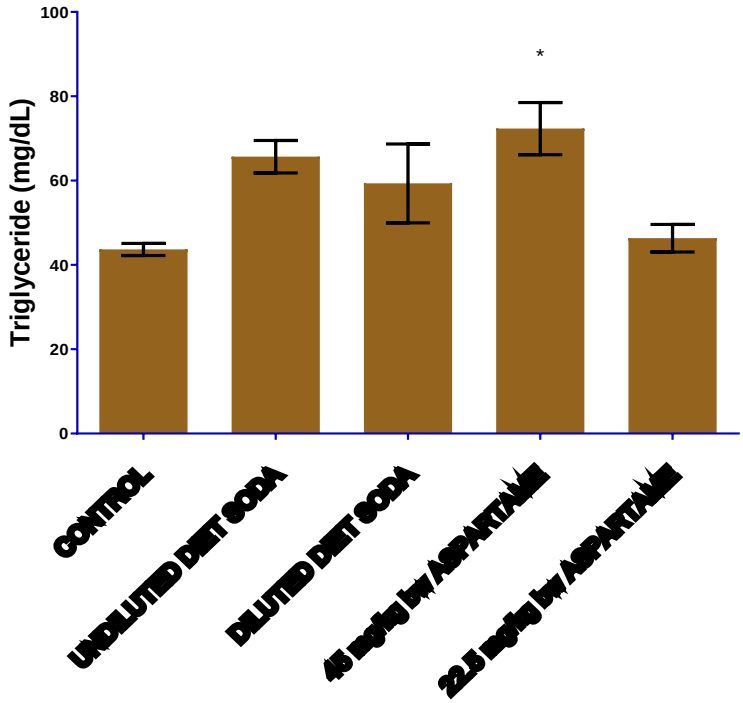

Figure IV: showing the effect of diet soda and aspartame on triglyceride (TG) concentration

*($P < 0.05$), results show there was a statistically significant increase in the conc. aspartame treated group compared with control ($P < 0.05$).

Figures V and VI compare mean values of plasma fibrinogen and viscosity of the various groups with control. There was a significant increase ($P < 0.05$) in the group administered 45 mg/kg b.w when compared with control.

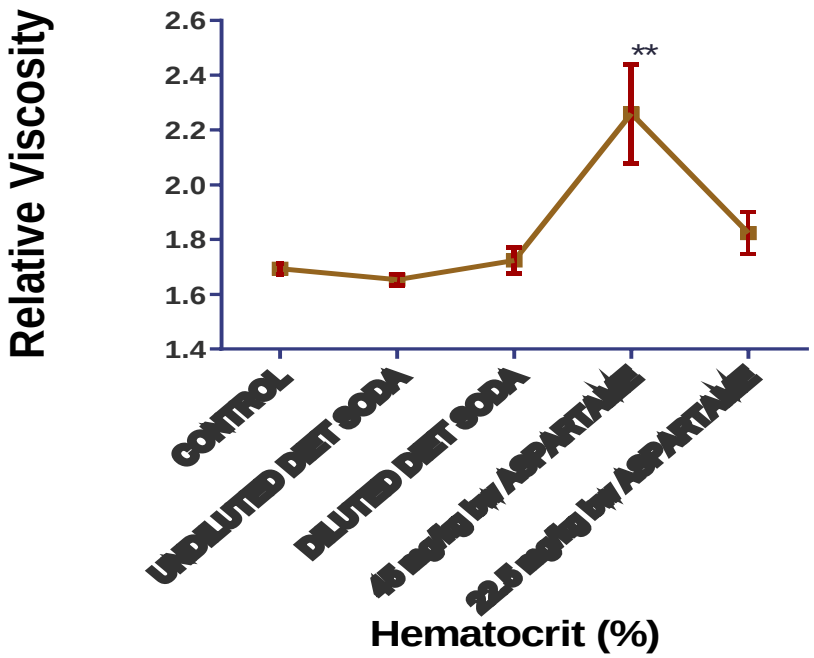

Figure V: a line graph showing the effect of diet soda and aspartame on relative viscosity of rats. ** $P < 0.01$), results show there was a statistically significant increase in the conc. aspartame treated group compared with control ($P < 0.01$).

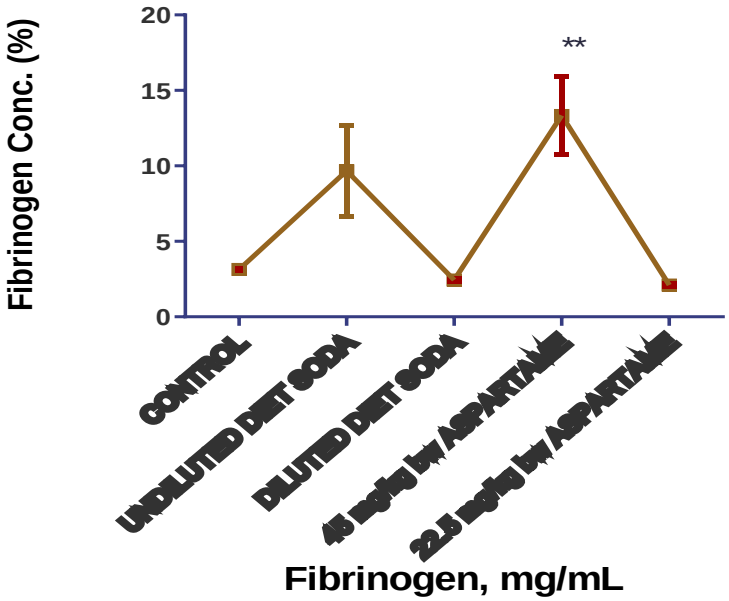

Figure VI: a line graph showing the effect of diet soda and aspartame on fibrinogen concentration in rats. ** $P < 0.01$), results show there was a statistically significant increase in the conc. aspartame treated group compared with control ($P < 0.01$).

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DISCUSSION

Most cardiovascular diseases can be prevented by addressing behavioral risk factors such unhealthy diet, obesity and physical inactivity. The effects of behavioral risk factors may show up in individuals as raised blood pressure, raised blood glucose, raised blood lipids, overweight and obesity (WHO 2019).

Findings in this study showed there was a significant increase in total cholesterol, triglyceride and LDL-C and reduction in HDL-C in rats administered high dose of aspartame (45 mg/kg b.w) while groups administered undiluted diet soda showed a significant increase in LDL-C only when compared with control. These findings are in agreement with report of Oluwatosin and Olubukola (2016), who observed an increase in TC, LDL-C, triglyceride and a reduction in HDL-C after administering 35 and 70 mg/kg b.w to rats for 9 weeks. In a prospective NutriNet-Santé cohort carried out by Charlotte et al. (2022) they suggested a potential direct association between higher artificial sweetener consumption (especially aspartame, acesulfame potassium, and sucralose) and increased cardiovascular disease risk. A meta-analyses study carried out suggest associations between artificially sweetened beverages and metabolic syndrome (Narain et al. 2017 and Zhang et al. 2021), artificially sweetened beverages were associated with increased risk of metabolic syndrome (Dhingra et al. 2007, Crichton et al. 2015 and Ferreira-Pêgo et al. 2016).

Low density lipoprotein cholesterol (LDL-C) is the predominant cholesterol-carrying lipoprotein, and is considered to be the main atherogenic lipoprotein. However other lipoproteins such as (HDL-C or very lowdensity lipoprotein have shown repeatedly to play a role in atherogenesis (Castelli 1988 and Barter et al. 2007. Recent epidemiological data suggests that isolated low HDL-C in people with normal LDL-C and triglyceride (TG) levels is equivalent to elevated LDL-C as a coronary risk factor (Lamarche et al. 1995, Goldbourt et al. 1997, Assmann et al. 1998 and Oluwatosin & Olubukola 2016)

The increase in TC, LDL-C Triglyceride and reduction in HDL-C confirms that aspartame consumption can predispose one to CVD and its consumption should be discouraged as its safety is questionable.

We also observed a significant increase in plasma viscosity and fibrinogen in groups that were administered 45 mg/kg b.w when compared with control. Blood viscosity is resistance to blood flow due to friction of the lamina that moves along the axis of the blood vessel due to differences in speed (Rosencranz & Steven, 2006). Several factors can affect blood viscosity including hematocrit, erythrocyte aggregation, erythrocyte deformability, fibrinogen levels, age, smoking, DM, dyslipidemia and others (Chen et al. 2012 and Irace et al. 2014). Fibrinogen is a facilitator of the formation of rouleaux through receptors on the erythrocyte membrane. It is a factor that determines changes in blood and plasma viscosity, and affects the aggregation of erythrocytes, which causes blood hyperviscosity.

Fibrinogen plays an important role as a medium for the interaction of erythrocyte and platelet cells, so that if fibrinogen levels increase, there will be an increase in erythrocyte aggregation and an increase in platelet aggregation, which often occur in acute stroke. The effect of fibrinogen on blood viscosity has been widely studied. In one study, three groups of patients were divided based on fibrinogen levels (Matsuda & Murakami 1976) Blood viscosity was significantly associated with blood fibrinogen levels in each group. Increased blood viscosity was associated with an increase in fibrinogen levels and was more pronounced in the hematocrit group with a higher value (Matsuda &Murakami 1976). Increased values of

plasma viscosity and fibrinogen observed in rats administered 45 mg/kg b.w which is within the allowable daily intake implies that aspartame can impair cardiovascular function even in the recommended daily dose.

CONCLUSION

In this study, there were increases in all the cardiovascular indices (TC, Triglyceride, LDL-C, and plasma fibrinogen and blood viscosity) assayed for. The increases were more prominent in groups that were administered the 45 mg/kg b.w and the group administered undiluted diet soda. There was no significant difference in all the parameters in rats administered low dose of ASP. The reduction in HDL-C which is protective to the CVS also confirms that aspartame can impact negatively on cardiovascular haemodymic factors. Even though this study was carried out in rats, there is need for caution to be applied in the consumption of aspartame and we recommend more detailed study to understand the mechanisms involved in the increases in the parameters assayed in this current study.

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