



Effect of non-nutritional Sweetener (Aspartame) on lipid profile in blood serum concentrations of Type-2 Diabetic Male Wistar Albino Rats

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Abstract: The present study aimed to express the worse effect of non-nutritional sweetener aspartame on streptozotocin (STZ) induced diabetic male Wistar rats. Aspartame was non-nutritional and low-calorie sugars which are nearly 200 times sweeter than table sugar sucrose. In this experiment we categorized four groups as control (C), Aspartame (ASP) (50mg/kg b.wt daily), Diabetic (D) (STZ single dose 45mg/kg b.wt) Diabetic+Aspartame (D+ASP) (STZ single dose 45mg/kg b.wt +ASP 50mg/kg b.wt daily). The blood serum concentration levels of total cholesterol (TC), triglycerides (TGs), very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) increased, and high-density lipoproteins (HDL) are decreased both in diabetic and aspartame administered rats. In this study, the serum levels of the above markers, which were highly increased in diabetes (D) and aspartame (ASP) consumed rats group when compared to control group rats. Therefore, Aspartame metabolites show negative effects on blood circulation, leading to stress-related disorders and dyslipidemia.

Keywords: Diabetes (STZ), Aspartame, Blood Serum levels, dyslipidemia.

I. INTRODUCTION

Diabetes mellitus is the most common heterogeneous and metabolic disorder in the world. More than 20 million people were affected by diabetes in India, and its number may be expected to increase by nearly 57 million by 2025 [1]. Type 2 diabetes is a common disease characterized by pancreatic beta-cell destruction and insulin resistance. [2]. Aspartame (methyl ester L-aspartic acid with L-phenyl alanine) is a widely used non-nutritional sweetener and have been using by individuals with diabetes and obesity. It is available in around 6000 food products such as beverages, cakes, chewable multivitamins, breakfast cereals, yoghurt type products and pharmaceuticals [3]. It provides as much as sweetness, nearly 200 times more than sucrose, and its energy value is calculated as 4 kcal/gm. Aspartame breaks down its metabolites in the gastrointestinal lumen as phenylalanine (50%), aspartic acid (40%) and methanol (10%) and produces various other metabolites, which will be concentrated in the blood circulatory system [4][5][6]. Among these metabolites, phenylalanine is converted into tyrosine in the liver and in the presence of the enzyme phenylalanine hydroxylase [7]. The epidemiological studies evidence that the intake of sugary beverages relates to harmful lipid levels, including fatty liver, cardiovascular disease, metabolic syndrome, and Type 2 diabetes mellitus [8][9][10]. Dyslipidemia in the type 2 diabetes is the cause for increased levels of total cholesterol, triglycerides, VLDL and LDL and decreased levels of HDL [11]. These kinds of obstacles comprise polyuria, polydipsia, polyphagia, retinopathy, ketosis and cardiovascular disorders. [12]. However, the literature about this study is meagre and needs investigation. The present study focused on identifying the lipoprotein levels and total cholesterol along with triglycerides. The impact of nonnutritive sweeteners in blood concentration alterations are observed through our experimental studies.

2. MATERIAL AND METHODS

2.1 Animals

Adult male Wistar albino rats, 8-10 weeks old, with an average body weight of 180-200g, were purchased from an authorized vendor (Sri Venkateswara Enterprises, Bengaluru, India) and used for all analyses. Rats were randomly selected and housed in polycarbonate cages with paddy husks as bedding, with free access to standard rat chow and tap water. Rats were acclimatized (temperature 25±2 °C, 12h dark/light cycle) for one week before being used in the experiment to adapt to the new environment. All experiments on animals were performed following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India (CPCSEA 2003). The protocol was approved by the Institutional Animal Ethics Committee (Regd. No. 438/01/a/CPCSEA/dt.17-07-2001) and have a resolution no. 10/(i)/a/CPCSEA/IAEC/SVU/ZOOL/SK/dt.08-07-2012.

2.2 Chemicals

Aspartame was purchased from Himedia Laboratories Pvt. Ltd. Mumbai, India and Streptozotocin was purchased from Sisco Research Laboratories Pvt. Ltd. Talaja, Maharashtra, India, and all other chemicals were of analytical grade.

2.3 Experimental design

Adult male albino rats 200-210 gms weight were divided into four groups (6n) and treated as given below:

Group – I: Control (C): daily tap water and feed were provided.

Group – II: Aspartame (ASP) administered: daily administered (via oral gavage for 30 days) with ASP (50 mg/kg body weight) dissolved in sterile saline.

Group – III: Diabetes (D): a single dose of streptozotocin (STZ) (45 mg/kg bodyweight).

Group – IV: Diabetes + Aspartame (D+ ASP): after considered diabetic rats daily administered (via oral gavage for 30 days) with aspartame (50 mg/kg body weight) dissolved in sterile saline.

2.4 Inducing diabetes with streptozotocin (STZ)

STZ dissolved in freshly prepared 0.1 M citrate buffer at neutral P^H. A single dose of STZ (45 mg/kg body weight) was given intraperitoneally after allowing the animals to fast overnight. After two days, animals were confirmed as diabetic, based on cut-made tail region and blood glucose levels observed with strips of Accucheck Glucometer.

Treatment with ASP was started on the third day after the STZ injection, which was considered the first day. ASP was orally administered as 0.9% sterile saline by the orogastric tube at a dose of 50 mg/kg bw for 30 consecutive days.

2.5 Animals sacrifices

After 30 days of the experiment, the rats were sacrificed by cervical dislocation, and the blood was collected into centrifuge tubes followed by separation of serum. Serum levels of FBS, Total cholesterol (TC), Triglycerides (TG), low-density lipoprotein (LDL), very-low-density lipoproteins (VLDL) and high-density lipoprotein (HDL) were assayed using a commercially available diagnostic kit (Span diagnostics, Surat, India).

2.6 Statistical analysis

The experiment was carried out in triplicates and expressed as mean \pm SD. To test the significance, one-way ANOVA was used among four different groups, followed by Tukey and Dunnet's multiple range test. This analysis was performed using a statistical program of social sciences (version 20; IBM SPSS Inc., Chicago, IL, USA). The data was regarded as significantly different at $P < 0.05$.

3. Results

Changes in the blood serum levels.

3.1 Total cholesterol

In the blood serum total cholesterol levels, significant changes were observed among Aspartame (ASP), Diabetic (D) and Diabetic+Aspartame (D+ASP) groups when compared with the control (C) group. Fig:1 Demonstrates the considerable difference between the groups (df 3 F= 117.667, $P < 0.05$).

3.2 Triglycerides

Triglycerides levels of serum significantly increased in the ASP, D and D+ASP groups when compared with the C group. Fig : 2 Demonstrates the significant change between the groups (df 3 F= 99.286, $P < 0.05$).

3.3 Very-low-density lipoprotein (VLDL)

The VLDL of serum levels is significantly higher in ASP, D, and D+ASP groups than in the C group. Fig : 3 Demonstrates the significant change between the groups (df 3 F= 49.095, $P < 0.05$).

3.4 Low-density lipoprotein (LDL)

LDL levels of serum significantly increased in the ASP, D and D+ASP groups when compared with the C group. Fig : 4 Demonstrates the significant change between the groups (df 3 F= 81.016, $P < 0.05$).

3.5 High-density lipoproteins (HDL)

HDL levels of serum significantly decreased in the ASP, D and D+ASP groups when compared with the C group. Fig : 5 Demonstrates the significant change between the groups (df 3 F= 49.449, $P < 0.05$).

4. Discussion

Diabetes mellitus is a most chronic disease in developing countries, and its severity is a public health problem in population growth and urbanization [13]. Diabetes induces most commonly by the STZ in rats; it leads to alkylation and death of pancreatic cells. This results in the reduction of releasing insulin [14]. In this study, 50 mg/kg body weight STZ was administered to raise the blood glucose levels in experimental rats. STZ destructs the pancreatic islets and β – cells death. Diabetes is mainly associated with multiple metabolic disorders among that lipid metabolism is affected by highly total cholesterol, triglycerides, VLDL, LDL and decreased levels of HDL are observed in many cases [15]. In the present study, diabetic rats lipid profile shows significantly increased levels of total cholesterol, triglycerides, VLDL, LDL and decreased levels of HDL compared with the control group rats.

Aspartame is potentially toxic and carcinogenic for humans, even takes as acceptable daily intake (ADI) [16]. Long term exposure to ASP leads to liver alterations and hepatocellular injury [17]. Elevated levels of LDL cholesterol are referred to as bad cholesterol; this leads to cholesterol storage in the arteries. This happens to increase the risk of stroke, heart diseases and atherosclerosis [18]. Non-nutritional sweeteners such as sucralose, saccharin and acesulfame K can suppress lipolysis and adipogenesis stimulation. In another study, reports suggest that few non-nutritional sweeteners like aspartame, saccharin and acesulfame K may cause to promote atherosclerosis through apolipoprotein A and HDL cholesterol impairments [19] [20]. The present study results explain that aspartame acts as a chemical stressor in diabetes mellitus diseased rats and control group rats. Its metabolites accelerate oxidative stress due to lipid peroxidation. Methanol is the causative for these factors. In the present investigation, significantly increased levels of total cholesterol, triglycerides, VLDL, LDL and decreased levels of HDL in Aspartame (ASP), Diabetic (D) and Diabetic+Aspartame (D+ASP) groups compared with the control (C) group rats. In the ASP and D groups, serum levels were moderately increased the total cholesterol, triglycerides, VLDL, LDL and decreased the levels of HDL over the control group.

5. Conclusion

The present study demonstrated the effects of non-nutritional Sweetener aspartame on diabetes and non-diabetic rats. Moreover, Aspartame consumption is more in diabetic individuals, and awareness in common people is needed regarding artificial sweetener usage. Further studies are required to elucidate the mechanisms involved in the development of these effects.

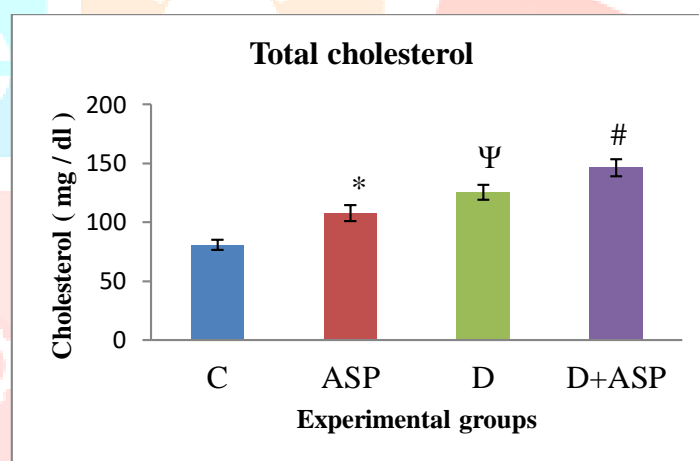


Fig. 1. Total cholesterol in the experimental groups at the end of the experimental period. Data are presented as mean \pm standard deviation. * Aspartame (ASP); Ψ Diabetes (D); # Diabetic+Aspartame(D+ASP) groups data indicate significant difference ($p < .05$), when compared with control (C) group.

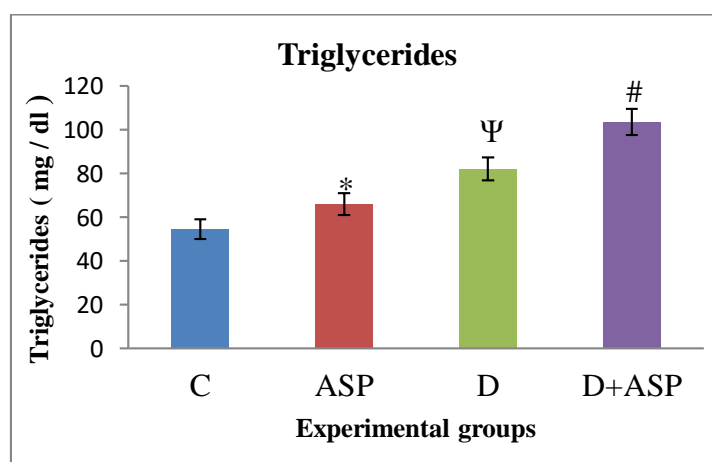


Fig. 2. Triglycerides in the experimental groups at the end of the experimental period. Data are presented as mean \pm standard deviation. * Aspartame (ASP); Ψ Diabetes (D); # Diabetic+Aspartame(D+ASP) groups data indicate significant difference ($p < .05$), when compared with control (C) group.

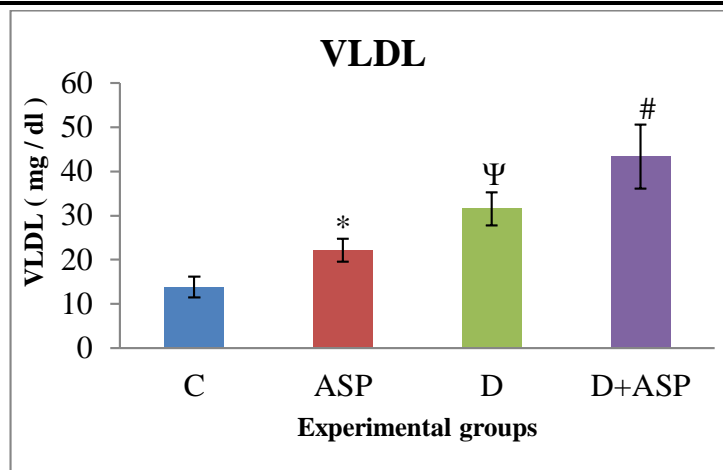


Fig. 3. VLDL profile in the experimental groups at the end of the experimental period. Data are presented as mean \pm standard deviation. * Aspartame (ASP); Ψ Diabetes (D); # Diabetic+Aspartame(D+ASP) groups data indicate significant difference ($p < .05$), when compared with control (C) group.

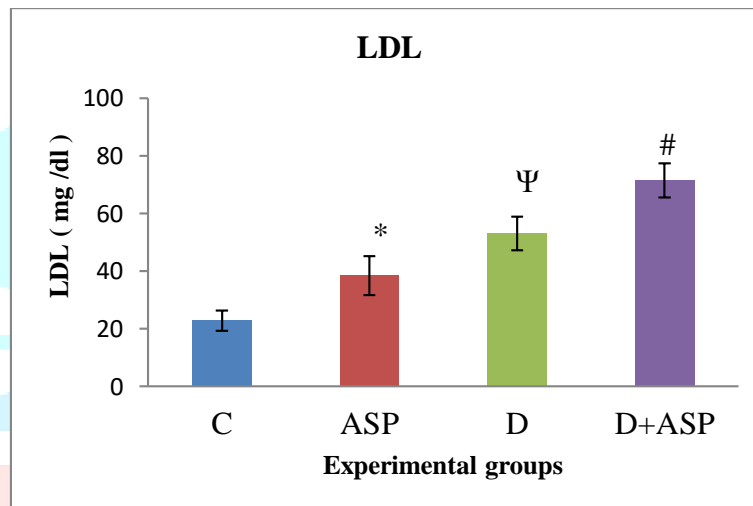


Fig. 4. LDL profile in the experimental groups at the end of the experimental period. Data are presented as mean \pm standard deviation. * Aspartame (ASP); Ψ Diabetes (D); # Diabetic+Aspartame(D+ASP) groups data indicate significant difference ($p < .05$), when compared with control (C) group.

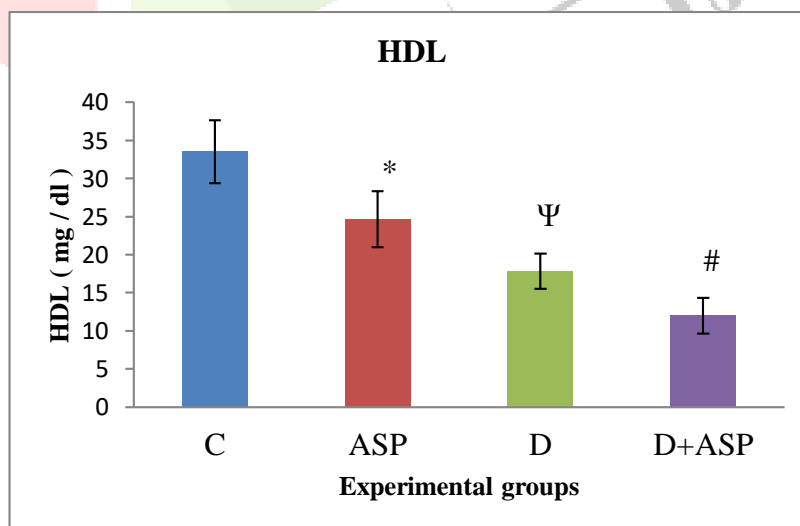


Fig. 5. HDL profile in the experimental groups at the end of the experimental period. Data are presented as mean \pm standard deviation. * Aspartame (ASP); Ψ Diabetes (D); # Diabetic+Aspartame(D+ASP) groups data indicate significant difference ($p < 0.05$), when compared with control (C) group.

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