Effects of *Stevia Rebaudiana* On Serum Lipid Profile of Sprague Dawley Rats

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Abstract

Background/Objective: The rising prevalence of cardiovascular diseases and obesity has prompted researchers to seek natural alternatives instead of synthesized sweeteners. Among the alternatives, *Stevia Rebaudiana* commonly known as *Stevia*, has emerged as a promising natural sweetener due to its intense sweetness without significant caloric content. There is need to examine its effect on serum lipid profile (total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TG) and very low density lipoproteins (VLDL)) in order to assess its impact on the parameters. This experiment study evaluated the effects of *stevia* on lipid profile of Sprague-Dawley rats. **Materials and Methods:** Thirty-two (32) adult male Sprague-Dawley rats were randomly separated into eight groups (4 per group). Control groups received normal chow and water for 14 days (acute) and 45 days (chronic) observation. Groups 3 to 5 and groups 6 to 8 received 4 mg, 8 mg and 12 mg per kg body weight of Stevia for 14 days and 45 days respectively by orogastric delivery. Animals were anaesthetized with chloroform. Blood samples and serum were collected by cardiac puncture for lipid profile assays by spectrophotometry. **Results:** There was significant increase in body weight for group 8 rats given 12 mg/kg ($p< 0.05$). There was significant increase in HDL level in group 8 when compared with acute group 8 rats given 12mg/kgbwt stevia ($p< 0.05$). There was a significant increase in LDL level the chronic group 8 that received 12mg/kg ($p< 0.001$). The findings from this study showed that chronic intake of stevia may stimulate weight gain, increased serum HDL-C level and increased serum LDL Regression analysis between body weight and serum LDL during 45 days (chronic) dosing showed a positive correlation which was not significant. **Conclusion:** These findings in animal experiment may suggest similar features in humans and require more investigations.

Keywords: Effects, Stevia, Lipid profile, Sprague Dawley rats

Introduction

The current rise in prevalence of cardiovascular diseases and obesity has attracted attention to explore natural alternatives to traditional sweeteners, such as sucrose and fructose. Food Sweeteners are chemical compounds found in nature and chemically synthesized, which have a sweet taste that determines their usage as sweetening agents. They are regularly used as sugar substitutes and most of them are not absorbed by the human body. They can be classified due to their origin (natural or synthetic agents), texture (powder and syrup), and caloric and non-caloric (1). *Stevia rebaudiana*, commonly known as Stevia, has emerged as a promising candidate due to its intense sweetness without contributing to caloric intake. This natural sweetener derives its sweetness from steviol glycosides, which are non-caloric compounds extracted from the *Stevia* plant. Most artificial sweeteners, such as cyclamates and saccharine, have been observed to be high calorie sugars and are potential carcinogens (2). Aspartame is another sweetener, proven to increase total cholesterol (TC), low density lipoprotein (LDL), triglycerides (TG) but reduces high density lipoprotein (HDL) (3).Increase in plasma

viscosity and fibrinogen concentration has also been associated with intake of aspartame in rats (3). However, *Stevia rebaudiana* has been shown to possess numerous therapeutic properties such as antihypertensive, anti-obesity and anti-hyperglycemic effects (4). Stevia extract is an alternative to synthetic sweeteners being approximately 200 to 300 times sweeter than sugar (5). As the popularity of *Stevia* consumption increases, it is important to examine the effects on various cardio-metabolic parameters. This study therefore, nvestigates the effects of *Stevia Rebandiana* sweetener on the levels of lipid profile panel in Sprague-Dawley rats. Serum Lipid profile commonly refers to the concentrations of TC, TG, VLDL, LDL and HDL in serum. Abnormal lipid profiles, characterized by elevated TC, TG, VLDL, LDL, and decreased HDL are associated with increased risk of atherosclerosis and coronary heart disease (6).

Materials and Methods

Thirty two (32) Sprague-Dawley rats that weighted 150-200g were separated in to eight different cages (four rats per group) in the Department of Pharmacology animal house and allowed to acclimatize for two (2) weeks (allowed standard rat chow and water *ad libitum*) under standard environmental condition of 12 hours light and 12 hours dark cycle with adequate ventilation. These were in accordance with the guidelines of National Research Council for care and use of laboratory animals (7). *Stevia Rebandiana* sweetener was procured as raw packaged Stevia, product of Cumberland Packaging Corp Brooklyn NY11285, USA. It was weighed and dissolved in distilled water and prepared in stocks for doses of 4 mg/kg, 8 mg/kg and 12 mg/kg body weight.

The rats were weighed after acclimatization, and randomly distributed in cages labelled groups 1to 8 as follow: Group 1 rats were the control group acute administration (14 days) given only normal rat chow and distilled water daily. Group 2 rats were control group for chronic administration (45 days) given only normal rat chow and distilled water daily.

Groups 3 to 5 rats were given Stevia orally for 14 days (acute administration) at the doses of 4 mg/kgbwt, 8 mg/kgbwt and 12 mg/kgbwt (respectively) in addition to normal rat chow and water. Groups 6, 7and 8 rats were given *Stevia* orally for 45 days at the doses of 4 mg/kgbwt, 8 mg/kgbwt and 12 mg/kgbwt respectively in addition to normal rat chow and water. At the end of 14 days and 45 days administration of *Stevia*, each rat was weighed, anaesthetized with 30 seconds to 1 minute inhalation in chloroform chamber. The thorax was dissected for collection of 4 to 5mL venous blood by right ventricular cardiac puncture. The blood was delivered into plain specimen containers and allowed clot until retraction appeared. The serum sample was collected and centrifuged at 5000 rpm for 5 minutes, serum decanted into plain collection tubes and stored frozen in refrigerator for lipid profile analysis.

The lipid profile were assayed as follows; TC enzymatic colorimetric method (8) , HDL, LDL and VLDL colorimetric precipitation methods(9) and TG by enzymatic colorimetric method (10).

Statistical Analysis

Statistical analysis was done with SPSS version 22. All the data were summarized as Mean ± SEM and subjected to further statistical analysis; using T-test for paired data and one-way ANOVA to identify the differences between the means for more than two groups. Regression analysis was used to estimate relationships between dependent and independent variables. A value of $P<0.05$ was accepted as significant difference.

Results

The results from this experiment revealed the following. There was progressive significant body weight increase ($p<0.001$) during 45 days (chronic) intake of Stevia associated with 4mg/kg to 12mg/kg doses (figure1). There was progressive significant increase in serum HDL occurred in rats given 12mg/kg bwt of Stevia for 45 days (chronic) against the acute or 14 days dosing ($p<0.05$) as shown in fig.2. There was significant reduction in serum LDL associated with only 12mg/kg bwt of Stevia during 14 days (acute) administration (fig3).

There was progressive significant increase in LDL across groups given Stevia for 45days ($p<0.001$), fig4. There was a progressive increase in body weight as serum LDL concentration increased during 45 days (chronic) administration of *Stevia*. The regression analysis showed a positive trend which was not significant. (fig 5).

Discussion

Stevia is a minimally caloric food sweetener derived from *Stevia* Rebaudiana (an aromatic shrub) known for its high sweetening index due to the glycoside contents such as stevioside and rebaudioside A (11). Lipid profile alterations (also associated with diets) play critical roles in the pathogenesis of cardiovascular and neurovascular diseases. It is still doubtful if adverse serum lipid alterations are associated with consumption of *Stevia*. This study investigated the effects of acute and chronic orogastric *Stevia* administration (4 mg/kg, 8 mg/kg and 12 mg/kg doses) on serum lipid profile of Sprague-Dawley rats in initial physiological condition. The results showed a statistically significant increase body weight for the group given 12 mg/kg of *Stevia* for 45days (chronic dosing). This may be due to stimulation of appetite (due to sweetening effect) that encouraged more food intake although differential food intake was not anticipated (and will be a subject for further investigation). Rebaudioside-A component in *Stevia* increases insulin production which may be responsible for body weight gain (). It may also be a direct effect on the tissues as the Stevioside component increases tissue insulin sensitivity and improves glucose transport into skeletal muscles (12). This finding of increase in body weight disagrees with a previous report that stevia consumption aided body weight control (13).

The search for alterations in blood lipids and lipoproteins as the major aim of this study revealed interesting results. There were no significant differences in TC during 14days (acute) and 45days (chronic) dosing of *Stevia*. This corroborated with a previous report which noted that stevia sweetener was not associated with TC changes (14). Triglycerides (TG) are common types of lipid that account for about 95% of all dietary fats. It is the body main source of energy and is essential for good health. High serum TGs are linked to higher risk of heart disease. This study did not show any statistically significant difference in TG across the groups for the doses of *Stevia*. This result agrees with a report which noted that *Stevia* extract did not stimulate increase on TG (15).

HDL are lipid protein complexes that mediate the transport of cholesterol from blood vessels and non-hepatic tissues to the liver for excretion. It is referred to as the "good cholesterol". In this study there was significantly higher HDL in chronic than acute dosing for 12 mg/kg of *Stevia* ($P<0.05$). This result is consistent with a previous study (14).

LDL as a carrier of cholesterol in the bloodstream unfortunately may deposit excess cholesterol in blood vessels to form plaques and atherosclerosis. It is otherwise referred to as the "bad cholesterol". In this study, there was significant decrease ($P<0.05$) in LDL- for 12 mg/kg *Stevia* against control during 14 days (acute) dosing (fig 3) as previously reported (16). On the contrary there was significant increase in LDL-($P<0.001$) during 45 days (chronic) dosing (fig 4). In our opinion this later effect of *Stevia* during chronic dosing cannot be explained in this study but there is tendency for adverse LDL elevation in chronic *Stevia* consumption. VLDL transports endogenous TG, phospholipids and cholesterol. It is also referred to as bad cholesterol because in excess, causes plaque formation in blood vessels. The reference value for VLDL is 30mg/dl. This study showed generally supressed VLDL values in both the acute and chronic administration of *Stevia* (figs 3and 4) as previously reported (15). In previous experiments, there were decrease in body weight gain, food intake, TC, TG and LDL concentration, and increase in HDL levels in rats fed high fat diet (for induction of obesity and hyperlipidaemia) and *Stevia* (17,18). In this study, the regression analysis revealed a positive correlation between body weight increase and serum LDL for chronic administration of *Stevia* (although statistically

not significant) but gives an insight into the prospective possibility for obesity and adverse increase in LDL-C for continuous use.

Conclusion

This study has pointed out the possibility that chronic Stevia consumption may induce progressive weight gain and tendency for increase in serum LDL which were contrary to previous reports. This study has also showed the ability of Stevia to increase HDL and suppress VLDL during chronic dosing. These findings in this animal experiment require further evaluation because they contrast with a previous report. These are particularly necessary for human concern who will consume Stevia on chronic basis.

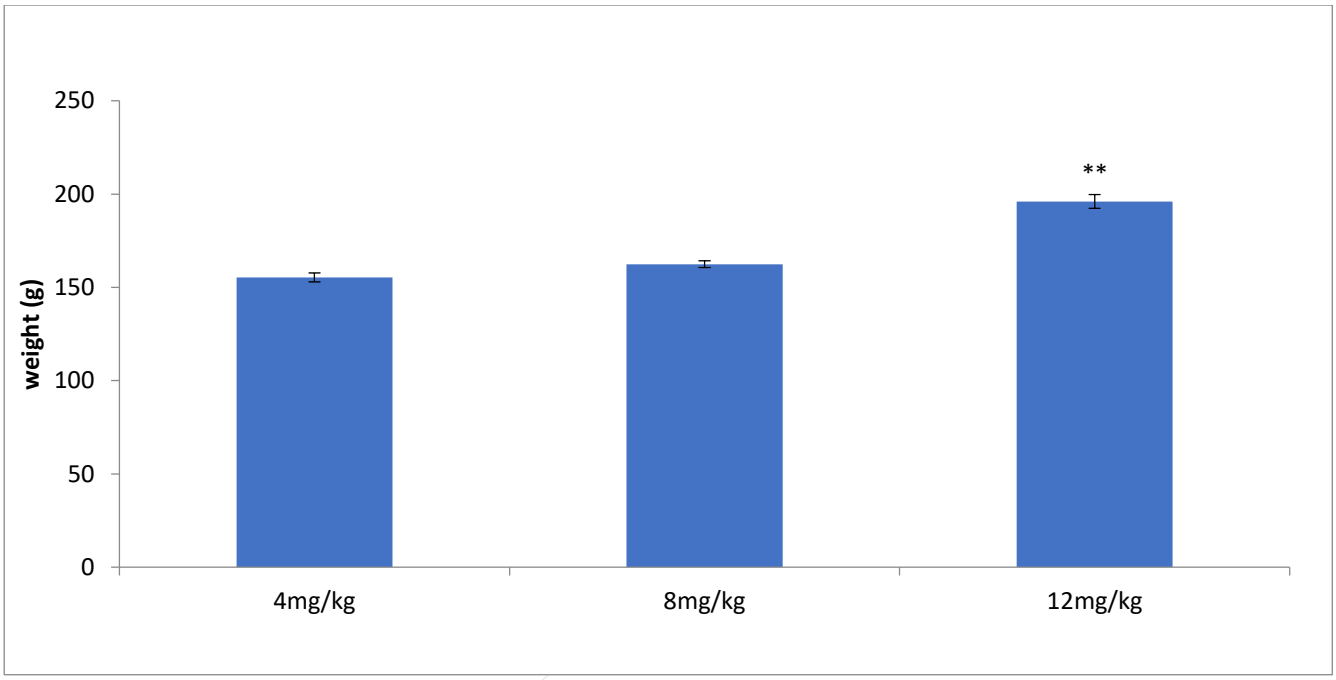

Fig1: Body weight showed significant increase i n rats given 45days dosing of 12mg/kg body weight of *stevia* compared to 4mg/kg and 8mg/kg (P<0.001).

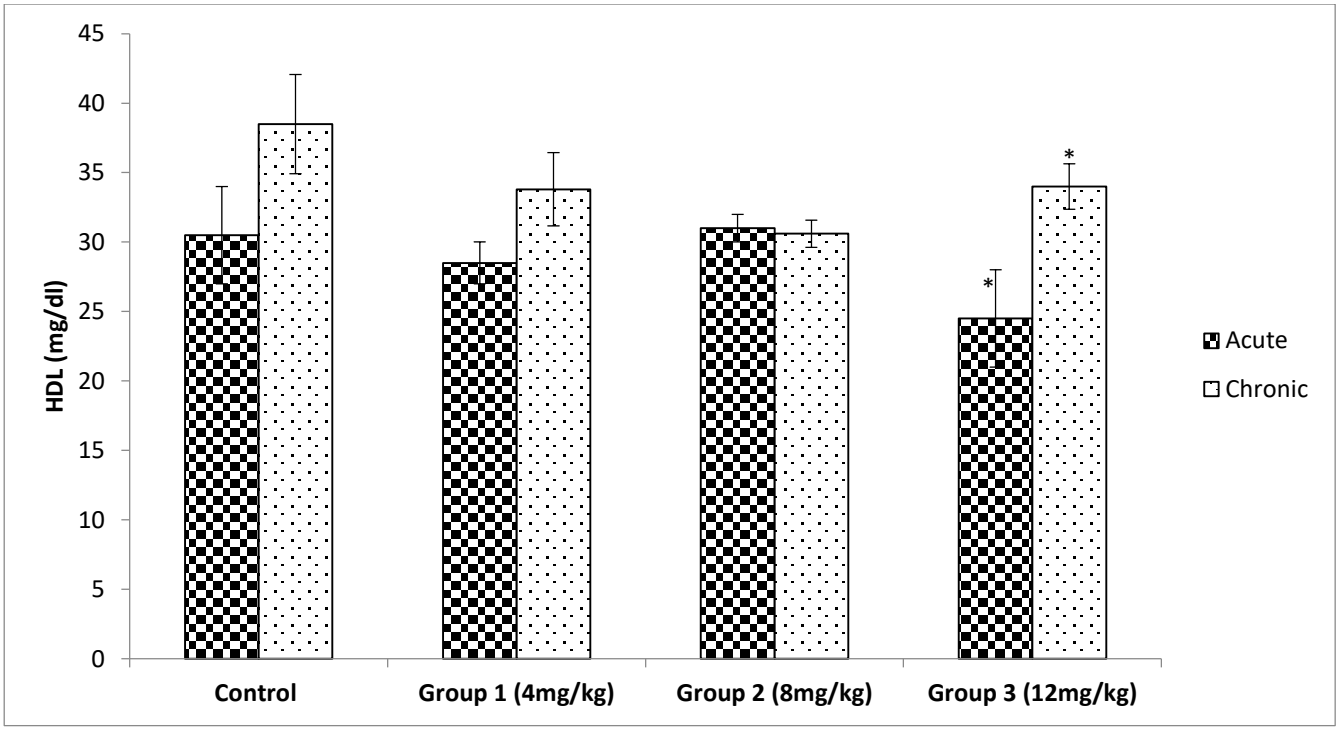

Fig.2: Showing statistically significant increase in only serum HDL for chronic against acute Stevia dosing for 12mg/kg bwt (P<0.05).

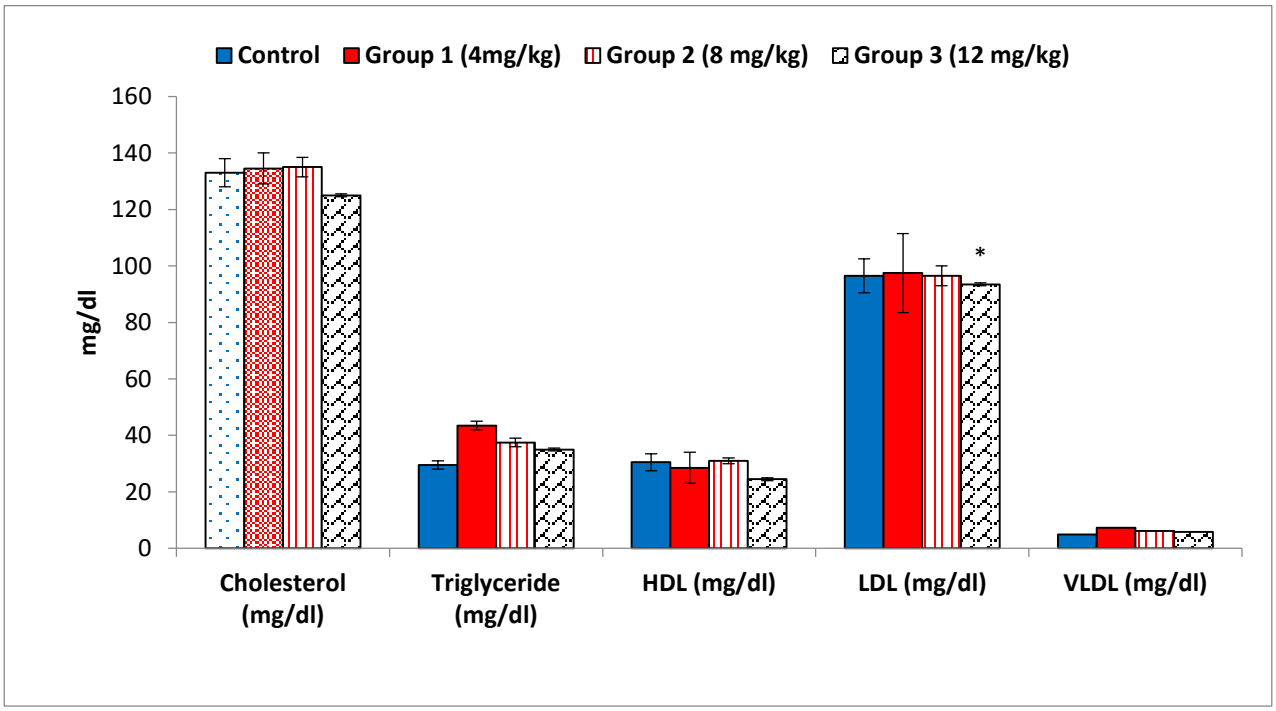

Fig.3: Showing statistically significant decrease in only LDL in group administered 12mg/kg (for acute or 14days) when compared with control (p<0.05).

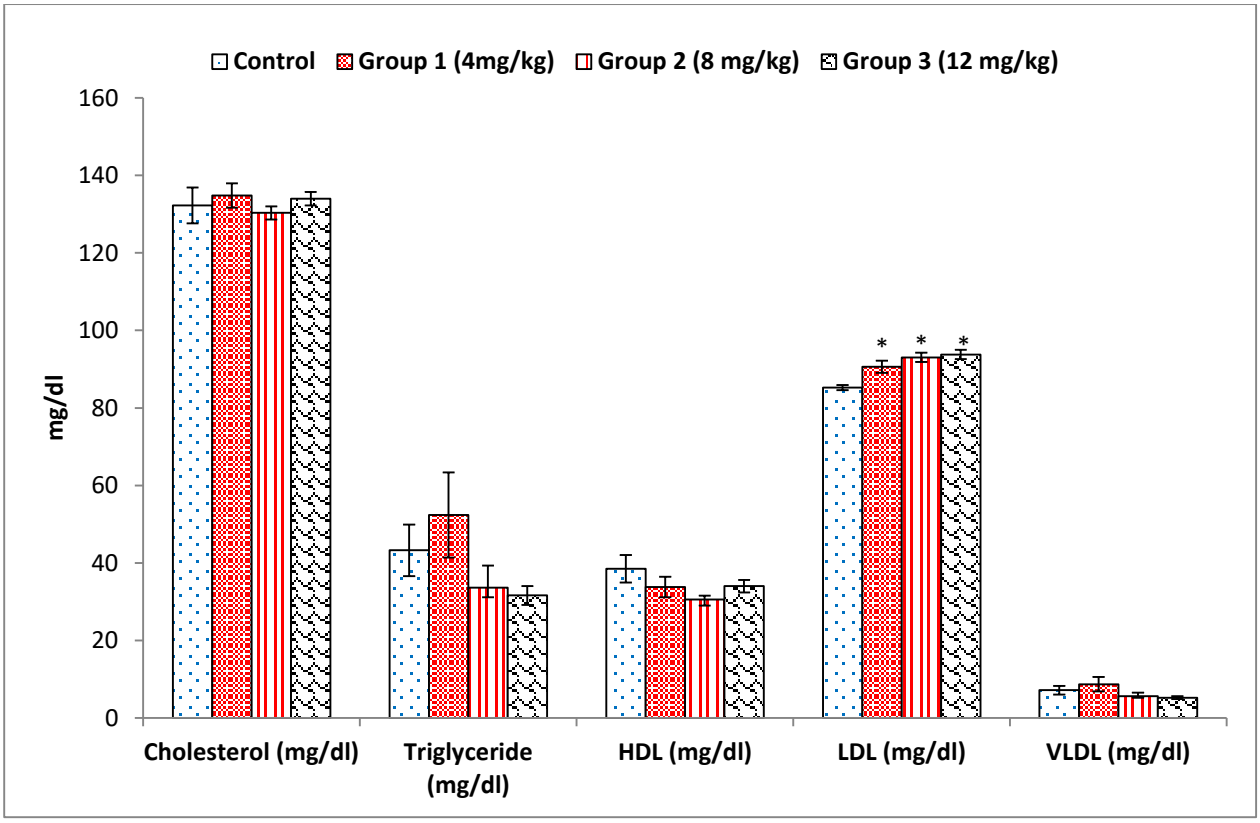

Fig.4: Showing progressive statistically significant increase in LDL only during 45 days (chronic) dosing of 4mg/kg, 8mg/kg, and 12mg/kg bwt of *Stevia* when compared with control (P<0.001).

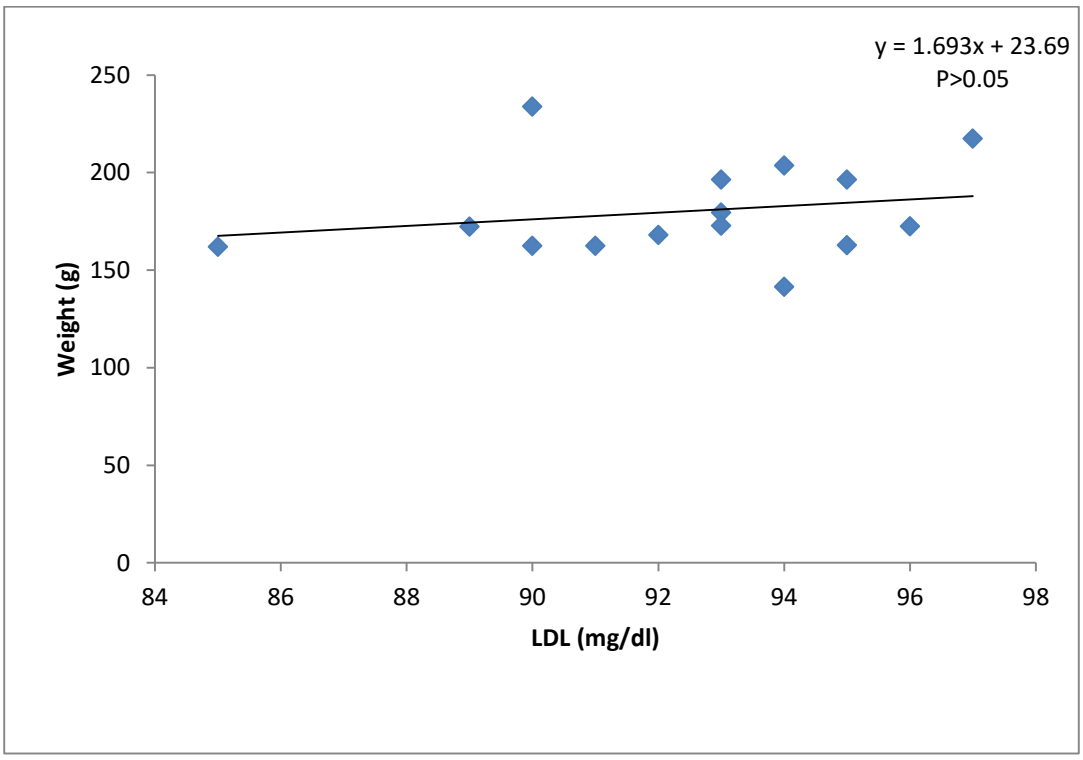

Fig 5: Regression graph of Body weight (g) vs LDL (mg/dl) during chronic administration of *Stevia* doses.

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