ORIGINAL ARTICLE

POTENTIAL RESTORATION OF FATTY LIVER ENZYMES WITH *STEVIA REBAUDIANA*

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Background: Obesity is a prevalent issue affecting a growing number of individuals globally. It is a systemic disorder with complications and co-morbidities. Non-alcoholic fatty liver disease (NAFLD) is a disastrous outcome of metabolic syndrome induced by obesity. Objective of this study was to find the effect of Stevia leaves in restoration of liver function tests (ALT, AST, ALP, and Bilirubin) in obese Sprague Dawley rats. **Methods:** This animal experimental study was carried out in the Physiology Department, in collaboration with the Pathology Department of Islamabad Medical and Dental College, and National Institute of Health, Islamabad on 90 healthy male Sprague Dawley rats over a period of 14 weeks. The animals were divided randomly into three groups of 30 rats each. Group 1 was given normal diet while Groups 2 and Group 3 were given high fat diet. Stevia leaves were further added for six weeks in the diet of Group 3. **Results:** High fat diet induced NAFLD in rats was ameliorated significantly on treatment with stevia. On comparison of liver function tests in obese control with stevia treated group, the values of ALT and AST were significantly decreased ($p<0.05$). However, ALP and bilirubin were not decreased significantly. **Conclusion:** *Stevia rebaudiana* exerts hepatoprotective effect in restoring liver damage due to high fat diet induced NAFLD in Sprague Dawley rats.

Keywords: *Stevia rebaudiana*, NAFLD, liver damage, obesity, liver function tests

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INTRODUCTION

Prevalence of obesity has considerably increased over recent years all over the world. It is a systemic disorder that predisposes individuals to various complications and co-morbidities like hypertension, hyperlipidemia, diabetes mellitus, atherosclerosis and fatty liver disease. Obesity can also lead to liver problems just like alcohol misuse and drug abuse which can alter the liver enzymes. Non-Alcoholic Fatty Liver Disease (NAFLD) is due to collection of triglycerides in the hepatocytes which can be identified with radiological as well as histopathological findings like steatosis, lobular inflammation, hepatocyte ballooning and fibrosis. NAFLD can progress into Non-alcoholic Steatohepatitis (NASH), fibrosis and eventually into cirrhosis which can lead to hepatocellular carcinoma. NAFLD causes increased serum liver enzymes. There are various causes of fatty liver-induced hepatitis. Potential pathophysiology for fatty accumulation can be:

1) Reduced β-oxidation of fatty acids by mitochondria[1]
2) Increased endogenous synthesis of fatty acids[2]
3) Enhanced fatty acid transport to the liver[3]
4) Diminished metabolism of very low density lipoproteins (VLDL) or triglycerides[2].

There is still ongoing research to find out causes and effects of NAFLD. A few recent studies show high levels of hedgehog pathway activation in patients with advanced fatty liver disease. The suggestion still indicated that the hedgehog pathway is an adult liver repair regulator.[4] In NAFLD there is also progressive procoagulants imbalance from inflammatory steatosis to cirrhosis. The Liver Function Tests (LFTs) detect liver enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) which are raised in the inflammation or damage of hepatocytes. Amongst different hepatic enzymes ALT has been associated with fatty liver disease which shows major role in metabolic syndrome. ALT is formed in liver cells and is also known as serum glutamic-pyruvic transaminase (SGPT). ALT is also used to evaluate the prognosis of liver disease.

To combat obesity, there has been a growing inclination towards substituting regular sugar with artificial sweeteners to decrease calorie consumption. However, the safety of artificial sweeteners has been a subject of debate since their discovery.[5] These substitutes have been associated with various health risks such as weight gain, potential carcinogenic effects, increased diabetes risk, and even reported links to depression.[6] Stevia, scientifically referred to as *Stevia rebaudiana* Bertoni, is a naturally occurring small perennial plant with sweet properties. Indigenous to the South American continent, Stevia is a commonly found shrub-like plant.[7] It belongs to the Asteraceae family of plants. Native people have been using the leaves of this plant since centuries in herbal teas for its sweet flavour and even used it in medicines. It was primarily introduced to other parts of the world by a Swiss botanist and naturalist named Moieses Santiago

Bertonie in year 1887 who learned the effects of Stevia from local Latin Americans.[8] Chemically the natural components of the stevia shrub is commonly known as stevioside or steviol glycosides which form the main characteristic sugary taste. In 1931 two French pharmacists isolated the steviols as steviosides extracts.[9] The main element constituting the sugary taste is stevioside present in stevia leaves. Japan was the foremost country to industrialize the commercial use of stevia in unpurified form as stevioside. Eventually the use of stevia and its extracts started commercially worldwide in health and food industries.[10] The effect of stevia as an anti-obesity, anti-diabetic and its help in controlling dyslipidemia has been reported.[11] The association of NAFLD with stevia treatment is still an ongoing process and very few investigations prove that stevia has effect on diminishing the NAFLD and there is requirement of further interventional researches to be carried out to explore the effects of stevia on NAFLD.[12]

The objective of this study was to see the effect of stevia leaves in restoration of some liver function tests in obese Sprague Dawley rats.

MATERIAL AND METHODS

This research experiment took place at the Physiology Department in collaboration with the Pathology Department of Islamabad Medical and Dental College, and National Institute of Health Islamabad after approval from College of Physicians and Surgeons Pakistan, over a duration of 18 months from Jul 2020 to Jan 2022. Ninety rats were selected for the study, and were divided into 3 groups of 30 healthy Sprague Dawley rats. The facilities provided for the animals complied with international standards.[13,14] The room was well ventilated and kept at controlled conditions of temperature (22–24 ºC). A 12-hour light and 12-hour dark cycle[9], was maintained in the animal house of NIH.[15] Animals were given water *ad libitum*.

Group 1 (n=30) was normal control on normal chow diet. Group 2 (Obese control, n=30) received a high fat diet with water during the entire study period. Group 3 (Obese on whole Stevia, n=30) was given dried, crushed Stevia leaves mixed with their food in a dose of 200 mg/Kg body weight, for a further period of 6 weeks adjusted weekly based on body weight.[16] At the end of the study period, blood sampling was done through intra cardiac puncture. The blood samples after clotting were centrifuged and serum was used to assess liver function tests (ALT, AST, ALP, and total bilirubin) using commercial kits on autoanalyzer (Selectra E fully automatic Chemistry Analyzer).

Statistical analysis was done on SPSS-24. The quantitative data like ALT, AST, ALP, and bilirubin were expressed as Mean±SD. One-way ANOVA and Post Hoc Tukey test were applied to see the differences between groups, and $p \leq 0.05$ was considered statistically significant.

RESULTS

One way ANOVA showed that there was statistically significant differences among the groups in ALT, AST, and Bilirubin ($p<0.05$), while ALP did not show significant differences ($p=0.063$) among the groups. (Table-1).

On comparison of liver function tests of normal group with obese control the differences between ALT, AST, and bilirubin were highly significant ($p<0.05$), while ALP was increased in obese control but this increase was not statistically significant ($p=0.163$). Comparison of normal group with stevia shows statistically significant differences in ALT only ($p<0.05$). On comparison of liver function tests in obese control with stevia group, the values of ALT and AST were significantly decreased ($p<0.0001$). The ALP and bilirubin were not decreased significantly ($p>0.05$) (Table-2).

Table-1: Comparison of serum ALT, AST, ALP, and Bilirubin among groups with one-way ANOVA

Parameter (Serum)	Normal control (n=30)	Obese control (n=30)	Stevia treated (n=30)	*p*
ALT(µ/L)	65.14±6.07	160.71±14.30	80.14±10.75	<0.001*
AST(µ/L)	53.86±6.09	111.57±23.94	61.00±6.66	<0.001*
ALP (µ/L)	302.71±31.7	332.0±18.0	333.85±31.17	0.063
Bilirubin (mg/dL)	0.79±0.16	1.00±0.12	0.91±0.11	<0.017*

*Significant

Table-2: *p*-Values for mean differences between groups on post-hoc Tukey's test

Groups Compared	Bilirubin	ALT	AST	ALP
Control *vs* Obese	0.027*	<0.001*	<0.001*	0.163
Control *vs* Stevia	0.287	0.046*	0.769	0.126
Obese *vs* Stevia	0.623	<0.001*	<0.001*	0.999

*Significant

DISCUSSION

Being overweight and obese is a major dilemma worldwide. Obesity is a major risk factor of cardiovascular disorders, metabolic derangements, carcinogenesis as well as musculoskeletal problems.[17] Non-alcoholic fatty liver disease (NAFLD) is a hallmark of obesity due to high calorie diet. Weight reduction is recommended to reverse the effects of NAFLD.[18] The most feasible way for obese people for caloric reduction is by adding non-nutritive sweeteners in the diet. Despite ongoing improvements in medicinal practices, safety of medicinal herbs and their effects is an active domain of research to treat fatty liver disease. As the novel researches prove that treating diseases with extracts are always more effective in treatment than herbs themselves.[19,20] Our effort was to find the effect of a herb *Stevia rebaudiana* on high fat diet inducted fatty liver in murine model. Liver is a sensitive organ and its

function can be assessed by measurement of raised liver enzymes, i.e., ALT, AST, ALP, and serum Bilirubin.[21] Our results demonstrated that there was a significant increase in liver enzymes in obese control rats which is consistent with recent work of Haung *et al*[22], who devised NAFLD model by inducing high fat diet to mice. They concluded that high fat diet can increase liver enzymes levels suggesting severe hepatocellular injury proved with their histological findings. Similar results were revealed in our study. The increase in level of liver enzymes in high fat diet induced rats is caused by formation of free radicals and protein glycosylation in the liver parenchyma.[23]

The ALT, AST, and even the bilirubin level were significantly decreased in our study to almost normal levels indicating hepatoprotective effect of the plant. The ALP and bilirubin that are markers of hepatobiliary tract and liver parenchyma, remained unaltered in stevia treated animals. Latha *et al*[24] compared protective effect of alcoholic extract of stevia leaves and stevioside in lipopolysaccharide induced liver injury of rats. They observed that these extracts of stevia significantly restored the raised ALT, and AST levels to normal. Our results of hepatic enzymes are consistent with Emam *et al*[25] who studied the effect of stevia in high fat diet induced diabetic rats and found that the parameters are significantly improved. AbdElwahab *et al*[26] showed no change in liver enzymes after stevia treatment compared with normal controls while aspartame caused significant elevation in ALT, AST, and even ALP. They concluded that altered hepatocellular function in metabolic syndrome reversed ALT by 62%, AST by 57% and ALP by 41% with administration of stevia leaves that was far better than results of aspartame. Our findings of significant restoration of liver enzymes are in disagreement to them. A possible reason for that may be their use of commercial alcohol treated extract of rebiana, while we used whole stevia leaves; and they made hepatotoxic model with alloxan while our rat model was high fat induced NAFLD.

Our results are controversial to similar study done by Ranjbar *et al*[27]. They studied the effects of stevial extract in high fat diet induced metabolic syndrome rats. They concluded that on administration of stevial extract the hepatic impairment deteriorates by increased ALT but no change in AST and ALP. Their results may be different from ours because they used different non-organic extracts of stevia and a commercially extracted rebiana with high extraction and purification quality[28], while we used dried whole leaves. Ranjbar *et al* also concluded that their regime dosage differences did not affect the amelioration of high fat liver changes.[27] The effect of different doses of stevia extract on LFTs was also compared by Elanga *et al*[29] by giving 25, 250, 500 and 100 mg/Kg/day dosage to female rats in groups. Their treatment with stevia extract showed comparable declension effects of liver enzymes by 500 and 1,000 mg dosage, while we used just 250 mg/Kg regime and got similar hepatoprotective effects. Our results proved that using whole leaf causes same reversal of not only ALT, and AST, but also ALP.

CONCLUSION

Stevia has hepatoprotective effect in high fat diet induced NAFLD which is exhibited by restoration of liver enzymes.

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