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ORIGINAL ARTICLE

Comparative Effects of Whole Stevia Leaf and Rebaudioside A on Oxidative Stress in Male Obese Sprague Dawley Rats

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ABSTRACT

Background: Obesity is a dilemma nowadays for increasing percentage of people worldwide. Obesity induced oxidative stress due to increased tissue lipid levels, threatened redox state of cell and increased free radical formation leads to tissue oxidative stress. Fatty liver disease is a very common disorder related with obesity resulting in storage of fat goblets in the liver tissues leading to altered redox state called oxidative stress.
Aim: To relate the outcome of Stevia and Rebaudioside A on Oxidative stress (MDA, SOD) in Obese Sprague Dawley Rats.
Place and duration of study: Physiology Department of Islamabad Medical and Dental College, Islamabad, in collaboration with National Institute of Health (NIH) from 1st January 2020 to 30th June 2022.
Methodology: One hundred and twenty healthy male Sprague Dawley rats were included. The animals were divided randomly into four groups of 30 rats each by simple randomization technique. Group 1 was given normal diet while the other three groups were given high fat diet. Stevia leaves and Rebaudioside A (rebiana) were further added for six weeks in the diet of Group 3 and 4 respectively. All animals were sacrificed at the end of study.
Results: High fat diet induced oxidative stress in obese group was restored to approximate normal values of SOD (0.001) and MDA (0.001) by treatment with stevia and rebiana both (0.899).
Conclusion: Stevia and Rebiana both cause improvement of oxidative stress unrewarding of expensive extraction procedures.
Keywords: Stevia rebaudiana, Rebaudioside A, Sprague Dawley rats, Oxidative stress, NAFLD

INTRODUCTION

Obesity prevalence has considerably increased over recent years all over the world. It is a systemic disorder that predisposes individuals to various complications and comorbidities like hypertension, hyperlipidemia, diabetes mellitus, atherosclerosis and fatty liver disease. Obesity can also lead to liver problems like alcohol misuse and drug abuse which can alter the liver enzymes. Obesity is also a principal cause of oxidative stress developed by fatty deposition in cells. Obesity induces oxidative stress by increased tissue lipid accumulation levels which threatens redox state of cell and increases free radical formation leading to tissue oxidative stress. Oxidative stress biomarkers are used in evaluation of status of disease and health improving effects of antioxidants. Oxidative stress due to obesity can be measured by level of lipid peroxidation marker like malondialdehyde (MDA) and decreased levels of tissue antioxidants enzymes like Glutathione peroxidase (GPx) and Superoxide dismutase (SOD). Distortion of oxidative equilibrium can lead to various liver disorders. It contributes in development of metabolic, inflammatory and proliferative liver disorders.[1] ROS are mainly formed in endoplasmic reticulum and mitochondria of liver cells in cytochrome P450 pathway enzymes. It is an ongoing process due to equilibrium between oxidants and antioxidants. The redox equilibrium is usually balanced under healthy conditions, cells have special enzymes to encounter it. The elements that are most effected by the ROS reactive agents in the hepatocellular structures are DNA, lipids and structural proteins. The process of oxidative stress is most likely related with many types of hepatocellular disorders, as well as monitoring oxidative stress can be a predictive marker for potential hepatic diseases and eventually can be used to research pharmacological interventions[2].

Lipid peroxidation causes release of proinflammatory products which are produced in progression of the liver disease. One of this lipid peroxidation product is malondialdehyde (MDA) which is a typical indicator of oxidative stress. MDA can also excite the liver stellate cells for increased production of collagen which can results in liver fibrosis. A rise in serum MDA is measured as parallel decrease of antioxidant levels in patients with NAFLD.[3] Levels of MDA were established to be significantly increased in patients with metabolic syndrome as well as NAFLD[4] which is now also challenged[5]. Serum MDA levels are reported to be remarkably increased in NAFLD than patients with viral hepatitis[6].

To reduce obesity, trend of using artificial sweeteners instead of normal sugar is increasing to minimize energy intake. Ever since the finding of artificial sweeteners their safety has been controversial. They have been reported to cause many health hazards like weight gain, carcinogenic, depression and increased diabetes risk. Stevia rebaudiana (Bertoni) is a herbal extract which is used nowadays as a natural sweetener[7]. Since its discovery it has been extensively researched for its safety and therapeutic properties. The natural sweet taste of this herb is due to the presence of certain active ingredients in the leaves that are Rebaudioside, Stevioside and steviobioside. However there are some differences between Stevioside and rebaudioside A. Stevioside has little less glucose moiety than Rebaudioside A (also known as Rebiana), Stevioside is also cost effective than rebaudioside A which is more expensive commercially. To predict toxicity of both natural sweeteners their toxicity should be studied individually to further recognize their safe use in human foods. Use of natural sweeteners is attractive for obese patients to reduce the calorie intake without the awareness of their side effects. To the best of our knowledge no such study has been performed that compared the effects of Stevioside and Rebaudioside on male obese rats. The present study was conducted to ascertain and compare the effects of natural sweeteners stevioside and rebaudioside A on liver function tests as well as oxidative stress markers MDA and SOD in hepatic tissue of male obese rats. This study will help in rationalizing the use of stevia as a whole instead of Rebiana extract and obese person can take cost effective stevia instead of buying expensive rebiana.

The study was conducted to compare the effect of both stevia leaves and its extract Rebaudioside (A) in order to establish their beneficial effects.

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MATERIALS AND METHODS

The study was carried out in the Physiology Department of Islamabad Medical and Dental College (IMDC), Islamabad, in collaboration with the Pathology Department of IMDC, and National Institute of Health (NIH), Islamabad after approval of synopsis by CPSP Research Advisory/Evaluation committee. The ethical approval for the experimental study was obtained from the Institutional Review Board (IRB) of IMDC, Islamabad. Total duration of the study was 2 years from 2020 till 2022. A total of 120 healthy male rats were included in the study, which were divided into four groups having 30 rats each.[1]Simple random sampling was done. The animal house was in accordance with international standards for breeding and housing of research animals.[8] The rats were housed 3 per cage, the size of which was in accordance with recommended standards (rectangular cage measuring 900cm^2 base and 23cm height).[9]

Rats were given water, ad libitum. Rodent feed normal and high fat diet was provided by the animal house of NIH. Groups 2, 3, and 4 were given a high fat diet (HFD) for 8 weeks to induce obesity, which was confirmed with calculation of Lee obesity index.[10] Dried Stevia leaves (200 mg/ Kg) and Purified Rebaudioside A (200 mg/Kg) were given to group 3 and 4 respectively along with high fat diet. The dosage of sweeteners was adjusted weekly based on body weight.[11] Sampling was performed at the end of the study period. Dissection was carried out for removal of liver and was placed in cold 25ml phosphate buffer solution (PBS).For sampling of MDA and SOD status in hepatic tissue homogenization of the organ was done and centrifuged at 1000rpm for 15 minutes. The supernatants were used for estimation of MDA and SOD activity according to Enzyme Linked Immunosorbent Assay (ELISA) kit protocol.

The statistical analysis was done by using SPSS-24. The statistical comparison between the groups was evaluated by one-way ANOVA and the comparisons between two groups were done by Post Hoc Tukey test. The difference was regarded statistically significant if the p-value was equal or less than 0.05.

RESULTS

There was a significant difference between the groups in MDA (0.001) as well as SOD (0.001). This difference of oxidative stress between the groups by measuring SOD and MDA is showed in Table 1.

There is a significant difference in values of normal control with all the other groups both in MDA and SOD. Stevia ameliorated the obesity associated raised MDA (0.008), as well as SOD (0.001) significantly. On comparison of obese group with rebiana group it concurrently shows significant decrease in MDA and increase in SOD. This depicts that both are good in decreasing the Oxidative stress (Table 2).

On comparison of stevia and rebiana group with each other there was not a significant difference neither in SOD (0.998) nor MDA (0.899). That suggests that the two sweeteners are equally effective in reducing oxidative stress. (Fig. 1).

Table 1 Comparison of superoxide dismutase (SOD) and malondialdehyde (MDA) among the groups with one-way ANOVA

Parameter	Control	Obese control	Stevia	Rebiana	P value
SOD (pg/L)	6.84±0.99	3.70±0.55	5.60±0.85	5.22±0.76	0.0001*
MDA(u/L)	71.96±2.59	94.93±2.94	86.56±5.45	86.33±6.75	0.0001*

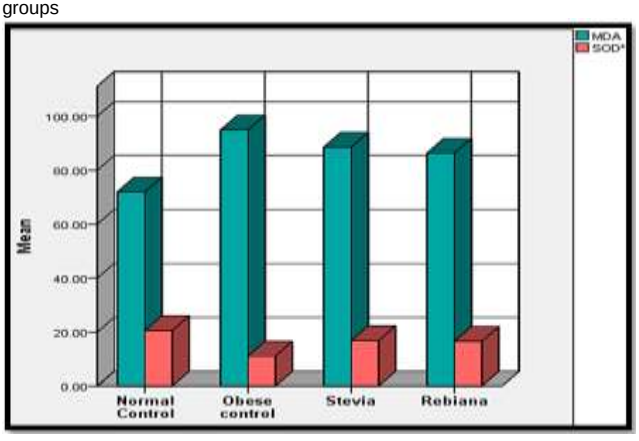
*p-value significant (< 0.05)

Table 2: Statistical differences of means of superoxide dismutase (SOD) and malondialdehyde (MDA) between the groups using post Hoc Tukey's test

Group Compared	SOD	MDA
Control vs Obese	0.0001*	0.0001*
Control vs Stevia	0.037*	0.0001*
Control vs Rebiana	0.025*	0.0001*
Obese vs Stevia	0.001*	0.008*
Obese vs Rebiana	0.002*	0.012*
Stevia vs Rebiana	0.998	0.899

*p-value significant (< 0.05)

Fig. 1: Representation of difference of oxidative stress markers between groups



DISCUSSION

Non-alcoholic fatty liver disease encompasses a wide range of manifestations like a parallel model for liver enzyme elevation also revealed oxidative stress imbalance. High fat diet induced oxidative stress can result in progression of multiple factors. Saturated fatty acids accumulation causes disturbed endoplasmic reticulum and mitochondrial homeostasis of oxidants and antioxidants balance.[2] .NAFLD induced by oxidative stress can be attenuated by anti-oxidant ability and anti-inflammatory moiety of stevia, which initiates, develops and restores hepatocellular damage[13].

Due to unclear evidence about stevia impact on fatty liver oxidative stress effects, we compared normal group with high fat diet induced Sprague Dawley rats and found a massive derangement in marker of lipid peroxidation, malondialdehyde MDA (p 0.001) accompanied with significant reduction (p 0.001) of key antioxidant superoxide dismutase (SOD).These findings unveil the harmful effect of high fat diet on hepatocyte oxidative stress.[14] In 2018, Sena et al[15] observed deranged MDA levels in high fat diet induced rats. More recent study in vivo conducted by Arya et al[5] concluded that MDA caused decreased SOD levels. They investigated the plasma levels of oxidative stress markers in human NAFLD patients and compared with healthy individuals. Based on molecular docking mechanism they concluded that MDA deactivates SOD in NAFLD patients[5].

This derangement of oxidative stress in obese group was tested by concomitant administration of stevia as well as rebiana. Our results depicted that there was statistically significant decrease in lipid peroxidation marker MDA as well as significant increase in antioxidant activity by measuring SOD. However, the antioxidative stress between stevia and rebiana was not statistically significant which means that they exert same activity despite having different flavonoid stir in them.

Our results about stevia effects on oxidative stress markers are consistent with many researches. It has been reported that it reduces hepatic steatosis as well as oxidative stress[16]. In 2010, Geerart et al[17] concluded that Stevioside treatment of 10mg/kg body weight improved the antioxidant levels in obese rats.

Complications in metabolic syndrome cause induction of cellular redox misbalances and lessening of antioxidants. Superoxide dismutase defends cell membrane by catalyzing superoxide ions into water and hydrogen peroxide. In 2013, Singh et al[18] conducted a study in alloxan induced diabetic rats to determine the effect of stevia on oxidative stress by homogenizing the hepatic tissue and measuring GSH and SOD. They claimed that SOD levels were normalized in stevia treated group which confirmed our results.

Stevia averts acute or chronic hepatocellular damage and oxidative stress by upregulation of Nuclear factor-erythroid related factor 2 (NRF2) as concluded by Ramos et al[19] in 2018. They conducted the study to affirm the effect of stevia on carbon tetrachloride induced toxic liver rat model and found out that stevia caused up regulation of NRF2 that contributed in oxidative stress control by preventing necrosis and cholestasis by modulating proinflammatory cytokines in experimental liver cirrhosis.

In 2018, Latha et al[20] designed a study to explore the protective effect of Stevial extract (500 mg/kg) and stevioside (250 mg/kg) in lipopolysaccharide induced acute liver damage in wistar mice. They found out that SOD increased with coinciding decrease in MDA levels revealing that both stevial extract and stevia inhibited oxidative stress.[3] This also confirms our result about oxidative stress in liver injury induced by high fat diet in NAFLD rats by giving small dosage of stevia of 200 mg/kg and rebiana (stevial extract) of 250 mg/kg.

Our result was also affirmed by Dong et al in 2020.[21] They also concluded that oxidative stress and inflammatory response are ameliorated by giving Rebaudioside A in vitro study. Further it was reported that Rebaudioside protected human hepatocytes from oxidative stress induced by CCL4 in culture.[22] These findings are consistent with our results but the difference lies in that we also recorded the effect of different forms of stevia. Another recent study in 2020 by Assi et al[23] obtained liver homogenate of diabetic rat models after treatment with stevia extract and found out that stevia ameliorated the raised MDA levels in diabetic murine model.

These results of decreased MDA and increased SOD activity by stevia in liver is explained by latest research of Isoyan et al[24] who concluded that stevia had superoxide producing lipoprotein fraction in its leaves which can moderate redox mechanism.

CONCLUSION

Stevia and Rebiana both cause improvement of oxidative stress irrespective of expensive extraction procedures.

Conflict of interest: Nil

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