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Phytochemical Characterization of Stevia and Its Hepatoprotective Effect in Rats

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Abstract

Stevia rebaudiana, a natural sweetener has long been utilized to promote liver function. The antioxidant and liver-protective properties of stevia were examined in this study in relation to rats' liver damage caused by carbon tetrachloride. To explore the phytochemical analysis of Stevia and to evaluate the hepatoprotective effect of Stevia in CCl4 induced hepatic injury rat model. The study was an experimental investigation carried out at the Food Analysis Laboratory, with the experimental procedures carried out in the rat facility at the Faculty of Allied Health Sciences, Riphah International University, Lahore Campus. In albino rats, the effects of Stevia, a non-calorific sweetener, on liver damage caused by carbon tetrachloride (CCl4) were assessed. Hepatotoxicity was induced by i.p administration of 30% CCL4 suspended in olive oil (1ml/kg Body weight). Animals were sacrificed at the end of the treatment period, and tests of antioxidant activity (FRAP, DPPH, TPC, and TFC) and biochemical markers (Lipid Profile, RFTs, LFTs, and Serum Glucose) were performed. Data analysis was done using MS-Excel and SPSS'25. The study examined four groups: the Normal, Diseased, Standard Drug, and Treatment group. The group that received Stevia treatment had notable hepatoprotective and antioxidant benefits. The phytochemicals found in Stevia, which are rich in antioxidants, lowered blood creatinine levels, stabilized protein and bilirubin levels, balanced liver enzyme levels (ALT, AST, and ALP), and greatly maintained liver function. Additionally, body weight, water and feed consumption, and other general health parameters were maintained by stevia. The findings suggested that Stevia may be able to prevent CCl4-induced liver damage if taken beforehand, and this effect is linked to Stevia's hepatoprotective properties.

Keywords: Stevia, natural sweetener, liver injury, hepatoprotective, carbon-tetrachloride**,** antioxidant

Vol.9, No.06; 2025

ISSN: 2581-3366

1. Introduction

Sugars play a vital role in the modern diet, appearing in large quantities in a variety of fruits, vegetables, and nuts in addition to being added as sweeteners to processed meals and drinks. These sweeteners are referred to as caloric sweeteners since they provide the diet with metabolized energy. Stevia rebaudiana (Bertoni) is a shrub plant native to South America. It has several common names such as candy leaf, sweet leaf, or sugar leaf [1]. Its leaves, stems, and inflorescences contain high-intensity sweeteners known as steviol glycosides, with an emphasis on stevioside and rebaudioside A, which are used as non-caloric sweeteners in diet and light food and drinks, replacing carbohydrates such as sucrose and fructose or artificial sweeteners. However, the existence of an "aftertaste" or bitter aftertaste is one factor that hampers the uptake of stevia sweeteners [2].

S. rebaudiana leaf extract is well-known for being 300 times sweeter than regular table sugar and has been used for millennia as a sweetener. Stevia is still a common food addition in the eastern countries [3]. Pretreating stevia leaves with ethanol is one method to lessen or completely eradicate the unfavourable taste of stevia products. Stevia leaves that have been pre-treated with ethanol (SLPE) can be used directly as an ingredient in food products that are meant to be sweetened and incorporate functional bioactives, like the SLPE sweetened cereal bar, or they can be used as a starting material to obtain stevia extracts with superior sensory quality [4].

Additionally, stevia's extracts, fractions, and isolated compounds, such as its glycosides, which are used as sweeteners and have useful qualities. In addition to being a sweetener, stevia has a wide range of phytochemicals, which are bioactive molecules that have drawn attention due to possible health advantages [5]. Stevia's phytochemical makeup is varied and includes glycosides (especially stevioside and rebaudioside A), diterpenoids, flavonoids, and phenolic acids, which give stevia its strong sweetness. These substances have a variety of biological functions, including antibacterial, hepatoprotective, anti-inflammatory, and antioxidant qualities, in addition to adding to the flavor [6]. The hypoglycemic and antidiabetic properties of stevia have been the most studied of its previously discovered qualities. These characteristics, along with the plant's ability to sweeten without adding calories, make it a natural product source of significant interest. It may encourage the development of novel foods or substances that may aid in the treatment of metabolic illnesses like diabetes mellitus (DM). In addition to natural sweeteners, terpenes, tannins, sterols, vitamins, flavonoids, carotenes, enzymes, organic acids, polysaccharides, hormones, and other complex mixtures of substances are found in stevia [7].

Studies have shown that S. rebaudiana glycosides and their extracts have pharmacological and therapeutic properties that include antibacterial, antioxidant, antihypertensive, anticancer, and antidiabetic activities [8]. Previous clinical investigations have demonstrated this plant's hypoglycemic and antihypertensive properties. These results supported the use of stevia by diabetic individuals who have a sweet tooth and experience negative effects from artificial sweeteners including cyclamate, aspartame, and saccharin. Since stevia is affordable and accessible to most consumers, it has the potential to be used extensively and may help people

Vol.9, No.06; 2025

ISSN: 2581-3366

control their weight because it has a beneficial influence on calorie substitution. Additionally, steviosides are utilized in a wide range of meals, drinks, medications, cosmetics, household chemicals, and other food-related sectors. Furthermore, administration of stevia leaf extract has been reported to result in non-significant toxicity [9].

Furthermore, stevia has a variety of therapeutic applications. Numerous ailments, including diabetes, obesity, hypertension, exhaustion, and depression, are treated with it [10]. In addition to increasing the body's ability to urinate, it has hypotensive, vasodilatation, taste-improving, sweetening, antifungal, antiviral, anti-inflammatory, antioxidant, and antibacterial qualities. Moreover, it has been shown in human tests to have positive effects on blood glucose and insulin levels, indicating a role in food intake management with no documented negative effects. There is evidence demonstrating that there is no histological damage to hepatic cells after Stevia rebaudiana leaf extract administration, confirming its preventive effect against liver damage [11]. Liver illnesses, which range from fatty liver disease to more serious problems including hepatitis and cirrhosis, pose a huge worldwide health burden [12]. With few therapy options available, there is a rising interest in studying natural substances with hepatoprotective properties. Stevia's extensive phytochemical profile makes it a good candidate for hepatoprotection [13].

Liver damage has a complicated pathophysiology that incorporates several different pathways. Numerous mechanisms, such as oxidative stress from toxic metabolites and immune-mediated injury marked by inflammatory responses, can cause direct hepatocyte destruction [14].

Liver impairment, particularly in the context of chronic liver diseases such cirrhosis and non-alcoholic fatty liver disease (NAFLD), has a substantial impact on renal function. The processes via which liver failure affects the kidneys must be understood in order to manage individuals who have both hepatic and renal issues at the same time [15]. Few artificial sweeteners are carcinogenic and have a variety of effects on normal liver function, yet researchers expressed concern about the usage of Stevia because they suspected it could be hazardous to the liver. Several preclinical researches have looked into the hepatoprotective properties of stevia extracts in animal models. These studies have proved stevia's capacity to alleviate liver damage caused by toxins, medications, and metabolic diseases. Despite these hopeful findings, the underlying mechanisms of stevia's hepatoprotective action are not fully understood [16].

Furthermore, while multiple researches have been conducted to investigate the phytochemical makeup of stevia, a thorough analysis that includes a wide range of phytochemicals found in various areas of the plant is required. Understanding the entire phytochemical profile of stevia is critical for determining its biological activity and medicinal potential. As a result, the purpose of this study is to look into the hepatoprotective impact of stevia in a rat model of carbon tetrachloride-induced liver injury, in order to better understand the underlying mechanisms.

This study seeks to gain a thorough understanding of stevia's potential as a hepatoprotective agent by combining phytochemical analysis with functional studies in animal models. The

Vol.9, No.06; 2025

ISSN: 2581-3366

study's findings may have significance for the development of novel therapeutic approaches for liver illnesses that use the natural substances found in stevia to promote liver health and general well-being.

2. Method

2.1 Study Design

The study design used for this research was experimental design.

2.2 Sample Size

This study included total 20 male rats which were divided into 4 groups, 5 each.

2.3 Study Duration

The study duration was 6 months after the approval of the research board. Trial period was 28 days.

2.4 Study Setting

The research took place in the Food Analysis Laboratory, where the experimental procedures were conducted in the rat facility at the Faculty of Allied Health Sciences, Riphah International University, Lahore Campus.

2.5 Preparation of Stevia Extract:

Stevia leaves were extracted using a hydroethanolic solvent system consisting of water and ethanol in a ratio of 70:30 (v/v). The leaves were macerated in the solvent mixture for 24 hours. The resulting extract was filtered to separate the liquid phase from the solid residue. The filtrate was then subjected to a water bath at 60°C to evaporate the ethanol. The remaining aqueous extract was preserved for further analysis.

2.6 Bio-efficacy Trial

Male Albino rats weighing 165g-200g were selected and housed in the animal facility at Riphah International University, Lahore, Punjab, Pakistan. They were observed for at least 10 days before experiment to ensure they were free from any infection. The animals were put into cages with well aired stainless steel covers at normal atmospheric temperature. And they were provided with normal diet and water (ad libitum). All this animal procedure was carried out under the supervision of Experimental Animal Ethics Committee.

2.7 Experimental Induction of Liver Injury:

30% of CCl4 was embedded in olive oil. CCl4 (1ml/kg) was induced in rats and caused liver injury.

Vol.9, No.06; 2025

ISSN: 2581-3366

2.8 Experimental Design

Rats were divided into 4 groups (n=5) i.e., normal control, diseased group, standard drug group, and treatment group. Normal control group was served as D0 in which rats were fed on normal diet. Diseased group was served as D1 in which rats were fed on normal diet and then administered with 30% CCl4 suspended in olive oil (1ml/kg), while D2 as standard drug group in which the rats were fed on standard drug i.e. Silymarin (100mg/kg) and then administered with CCl4. Treatment group was served as D3 in which the rats were fed on stevia (100mg/kg) and then administered with CCl4.

2.9 Test Analysis

For the 'Pre' and 'Post' assessment of blood samples of experimental rats during the study, Serum glucose level, Lipid Profile, Liver functioning tests, Renal functioning tests were used.

2.10 Antioxidants Activity Tests

To assess antioxidant activity of Stevia extract, TPC, TFC, DPPH, and FRAP were performed.

3. Results

3.1 Antioxidant and Phytochemical Activity

To assess stevia extract's antioxidant capacity and the existence of bioactive components, a phytochemical analysis was conducted utilizing a variety of techniques. A moderate to high ability to neutralize free radicals was indicated by the extract's 61.04% DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. This implies that the stevia extract has strong antioxidant qualities that may be essential in shielding cells from oxidative stress and halting damage from free radicals.

The extract's Total Phenolic Content (TPC) was determined to be 3265 mg/100 ml GAE. It is commonly known that phenolic chemicals have antibacterial, anti-inflammatory, and antioxidant properties. An elevated TPC value like this one suggests that stevia is abundant in phenolic chemicals, which greatly enhance its overall antioxidant capacity. Likewise, the Total Flavonoid Content (TFC) indicated a significant number of flavonoids, at 3790.50 µg/ml QE. Among its many health advantages, flavonoids, a significant class of plant-based antioxidants, lower the risk of chronic illnesses including liver disease.

Furthermore, the Ferric Reducing Antioxidant Power (FRAP) assay was used to assess the antioxidant capacity, and the results showed a value of 13.71 mM. This technique assesses the extract's capacity to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), another sign of antioxidant potency. The conclusion that stevia extract has a considerable reducing power and is useful in preventing oxidative stress is supported by the comparatively high FRAP value.

Vol.9, No.06; 2025

ISSN: 2581-3366

Table 1. Evaluation of Phytochemical and Antioxidant Activity of Stevia Extact

Methods	**Mean Value**
DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity	61.04%
Total Phenolic Content (TPC)	3265 mg/100 ml GAE
Total Flavonoid Content (TFC)	3790.50 µg/ml QE
Ferric reducing antioxidant assay (FRAP)	13.71 mM

3.2 Body Weight

The experimental groups D1, D2, and D3's starting and ending body weights are summarized in the table, along with statistical analyses to assess the significance of weight changes over the course of the study. By the end of the experiment, the mean body weight of Group D1 had risen from 185.20 ± 11.45 g to 200.40 ± 14.15 g. The observed increase was statistically significant ($p = 0.001$), with a 95% confidence interval for the mean difference spanning from -19.79 to -10.60 g. The standard error of the mean was 5.122.

The body weight of Group D2 increased significantly after the intervention, from an initial mean of 172.00 ± 6.67 g to 201.40 ± 6.06 g. With a 95% confidence interval of -35.76 to -23.03 g, the difference was extremely significant ($p < 0.001$) and the standard error of the mean was 2.983, suggesting that the treatment given to this group had a strong impact.

With an initial mean of 182.00 ± 9.69 g and a final mean of 208.40 ± 14.90 g, Group D3 also showed a notable increase in body weight. The statistical analysis showed a 95% confidence interval between -34.84 and -17.95 g, with a standard error of 4.335 and a p-value of 0.001.

Vol.9, No.06; 2025

ISSN: 2581-3366

Table 2. Comparison of Initial and Final Body Weights in Different Experimental Groups

Groups	Weight	Mean ± SD	Std. Error Mean	95% C.I of the Difference		df	Sig.
				Lower	Upper		
D_1	Initial Weight	185.20 ±11.45	5.122	-19.79	-10.60	4	.001
	Final Weight	200.40 ±14.15	6.329				
D_2	Initial Weight	172.00 ± 6.67	2.983	-35.76	-23.03	4	.000
	Final Weight	201.40 ± 6.06	2.712				
D_3	Initial Weight	182.00 ± 9.69	4.335	-34.84	-17.95	4	.001
	Final Weight	208.40 ±14.90	6.667				

3.3 Serum Glucose

In order to evaluate the metabolic impact of Stevia in the context of hepatoprotection, the serum glucose levels (mean ± SD) in groups D1, D2, and D3 are shown in the table both before and on Day 28. The mean glucose level in Group D1 (those receiving standard medication) rose from 74.20 ± 4.49 mg/dL prior to treatment to 81.40 ± 8.76 mg/dL on Day 28. Despite an increase, the difference was not statistically significant ($p = 0.140$), indicating that glucose levels were not significantly changed by the conventional treatment during the course of the treatment.

After 28 days, the glucose levels in Group D2 (Stevia-treated) increased from 69.20 ± 6.09 mg/dL to 83.60 ± 15.24 mg/dL. The fact that the shift was not statistically significant ($p = 0.185$) despite the apparent increase suggests that Stevia did not significantly alter serum glucose concentration, proving its non-diabetogenic nature, a crucial factor in hepatoprotective treatments.

In the same way, Group D3 increased from 70.20 ± 4.14 mg/dL to 85.00 ± 11.44 mg/dL, although this variation was likewise not statistically significant ($p = 0.091$). Even while the glucose levels in all groups showed an increased trend, the lack of significance indicates that these differences might be within physiological bounds and unrelated to hepatotoxic or hepatoprotective effects.

Table 3. Comparison of Pre and Post Serum Glucose Levels in Different Experimental Groups

Groups	Serum Glucose	Mean ± SD	Std. Error Mean	95% C.I of the Difference		df	Sig.
				Lower	Upper		
D_1	Glucose (Before)	74.20 ± 4.49	2.009	-18.07	3.67	4	.140
	Glucose (Day 28)	81.40 ± 8.76	3.919				
D_2	Glucose (Before)	69.20 ± 6.09	2.727	-39.42	10.62	4	.185
	Glucose (Day 28)	83.60 ± 15.24	6.816				
D_3	Glucose (Before)	70.20 ± 4.14	1.854	-33.37	3.77	4	.091
	Glucose (Day28)	85.00 ± 11.44	5.118				

3.4 Lipid Profile

Triglycerides (60.8 ± 2.31) and VLDL levels (12.8 ± 0.74) were substantially higher in the diseased group than in the normal group, suggesting liver injury. Comparable to the conventional treatment group, the Stevia-treated group demonstrated a significant decrease in both indices (VLDL: 8.3 ± 0.4; Triglycerides: 39.6 ± 1.01), indicating that Stevia has a protective effect on lipid metabolism.

While the treatment group (52.2 ± 3.54) maintained levels similar to the normal and standard medication groups, the diseased group's total cholesterol elevated significantly (67.6 ± 1.85), suggesting that Stevia successfully reduced CCl_4-induced hypercholesterolemia.

It's noteworthy to note that while Stevia kept HDL levels (30.8 ± 1.72) near normal, the diseased and conventional medication groups had higher HDL levels, indicating stronger physiological lipid regulation. The hepatoprotective effect of Stevia was further supported by the fact that LDL, a crucial indicator of liver dysfunction, was much higher in the diseased group (21.8 ± 1.16) but remained low in the Stevia treated group (11.8 ± 2.63), which was equivalent to the normal group.

Total lipid content increased considerably in the diseased group (396.2 ± 5.52) and decreased dramatically in the Stevia-treated group (346.2 ± 8.20), nearly matching the normal (347 ± 7.92) and standard medication (348 ± 10.5) groups.

Vol.9, No.06; 2025

ISSN: 2581-3366

Table 4. Lipid Profile Parameters Among Experimental Groups

Parameters	Groups	Mean ± SD	df	F	Sig.
Triglycerides	Normal	56.4 ± 13.3[b]	3	8.033	.002
	Diseased	60.8 ± 2.31[b]			
	Standard Drug	45.4 ± 2.15[a]			
	Treatment	39.6 ± 1.01[a]			
Cholesterol	Normal	51.8 ± 2.13[a]	3	15.696	.000
	Diseased	67.6 ± 1.85[b]			
	Standard Drug	46.8 ± 7.88[a]			
	Treatment	52.2 ± 3.54[a]			
HDL	Normal	30.0 ± 1.41[a]	3	7.716	.002
	Diseased	33.2 ± 0.74[b]			
	Standard Drug	34.0 ± 1.41[b]			
	Treatment	30.8 ± 1.72[a]			
LDL	Normal	12.4 ± 1.49[a]	3	32.308	.000
	Diseased	21.8 ± 1.16[b]			
	Standard drug	12.0 ± 1.09[a]			
	Treatment	11.8 ± 2.63[a]			
VLDL	Normal	9.0 ± 0.89[a]	3	31.404	.000
	Diseased	12.8 ± 0.74[b]			
	Standard drug	9.2 ± 0.74[a]			
	Treatment	8.3 ± 0.4[a]			
Total lipid	Normal	347 ± 7.92[a]	3	35.596	.000
	Diseased	396.2 ± 5.52[b]			
	Standard Drug	348 ± 10.5[a]			
	Treatment	346.2 ± 8.20[a]			

3.5 Liver Function Test

Stevia's hepatoprotective impact was assessed using liver function measures in a rat model of CCl_4-induced liver injury. The diseased group's ALT and AST levels were much higher (ALT: 684.8 ± 16.9; AST: 608.2 ± 21.8), indicating that CCl_4 had caused serious liver damage. The Stevia-treated group, on the other hand, demonstrated a considerable decrease (ALT: 385 ± 17.3;

Vol.9, No.06; 2025

ISSN: 2581-3366

AST: 415.4 ± 40.7), comparable to the standard medication group, suggesting that Stevia has a preventive effect against hepatocellular injury.

Although the increase was marginally greater in the treatment group, ALP was moderately high in the diseased group (314 ± 33.0) and further elevated in the treatment (382.2 ± 37.9) and standard drug groups. In contrast to untreated damage, the enzyme levels stayed within a reasonable range, which may indicate incomplete normalization of biliary function.

The group with the condition had a considerably higher level of total bilirubin (1.060 ± 0.13), a symptom of liver failure. Stevia's ability to mitigate cholestasis or decreased bilirubin clearance is demonstrated by the significantly lower bilirubin levels (0.780 ± 0.14 and 0.820 ± 0.07, respectively) in both the treatment and conventional medication groups.

Unexpectedly, the diseased group had higher levels of albumin and total protein (Albumin: 3.40 ± 0.08; Total Protein: 8.30 ± 0.26), which could be a sign of a compensatory hepatic response or changed protein metabolism after damage. Stevia's significance in sustaining protein synthesis and liver synthetic function was demonstrated by the near-normal restoration of albumin (3.10 ± 0.38) and total protein (7.16 ± 0.38) levels in the Stevia-treated group, which was comparable to the standard medication group.

Vol.9, No.06; 2025

ISSN: 2581-3366

Table 5. Liver Function Biomarkers Among Experimental Groups

Parameters	Groups	Mean ± SD	df	F	Sig.
ALT	Normal	44.8 ± 4.35[a]	3	1669.2	.000
	Diseased	684.8 ± 16.9[c]			
	Standard Drug	400.8 ± 7.24[b]			
	Treatment	385 ± 17.3[b]			
AST	Normal	43.4 ± 7.63[a]	3	218.76	.000
	Diseased	608.2 ± 21.8[c]			
	Standard Drug	426 ± 43.7[b]			
	Treatment	415.4 ± 40.7[b]			
ALP	Normal	253.8 ± 21.0[a]	3	10.916	.000
	Diseased	314 ± 33.0[b]			
	Standard Drug	362 ± 42.5[bc]			
	Treatment	382.2 ± 37.9[c]			
Bilirubin	Normal	0.460 ± 0.08[a]	3	18.708	.000
	Diseased	1.060 ± 0.13[c]			
	Standard drug	0.820 ± 0.07[b]			
	Treatment	0.780 ± 0.14[b]			
Albumin	Normal	3.18 ± 0.27[a]	3	3.663	.035
	Diseased	3.40 ± 0.08[ab]			
	Standard drug	3.62 ± 0.07[b]			
	Treatment	3.10 ± 0.38[a]			
Total Protein	Normal	7.26 ± 0.44[a]	3	9.179	.001
	Diseased	8.30 ± 0.26[b]			
	Standard Drug	7.56 ± 0.21[a]			
	Treatment	7.16 ± 0.38[a]			

3.6 Renal Function Test

Urea levels were significantly higher in the diseased group (59.8 ± 8.3), which suggests renal stress or poor nitrogen metabolism brought on by liver failure. In addition to having higher urea than the normal group (40.6 ± 7.2), the Stevia-treated group (52.2 ± 2.5) and the conventional

Vol.9, No.06; 2025

ISSN: 2581-3366

medicine group (52.8 ± 4.4) also had significantly lower urea than the sick group, indicating that Stevia provided some renal protection.

The diseased group also had higher levels of creatinine (0.88 ± 0.07), a more direct indicator of kidney filtration efficiency that may indicate renal involvement after liver injury. Nonetheless, the Stevia treated group (0.64 ± 0.10) kept creatinine levels around normal (0.54 ± 0.04) and much lower than the group using standard medication (0.80 ± 0.08), suggesting that Stevia pretreatment improved renal function preservation.

Table 6. Renal Function Biomarkers Among Experimental Groups

Parameters	**Groups**	**Mean ± SD**	**df**	**F**	**Sig.**
Urea	Normal	40.6 ± 7.2[a]	3	6.843	.004
	Diseased	59.8 ± 8.3[b]			
	Standard Drug	52.8 ± 4.4[b]			
	Treatment	52.2 ± 2.5[b]			
Creatinine	Normal	0.54 ± 0.04[a]	3	14.283	.000
	Diseased	0.88 ± 0.07[b]			
	Standard Drug	0.80 ± 0.08[b]			
	Treatment	0.64 ± 0.10[a]			

3.7 Water and Feed Consumption

The diseased group consumed the most water (149 ± 10.8 ml), most likely as a result of metabolic abnormalities and potential dehydration or compensatory reactions linked to liver damage caused by CCl_4. Although it was somewhat less than the diseased group, the conventional medicine group likewise displayed an elevated intake (142 ± 9.3 ml).

The Stevia-treated group, on the other hand, consumed the least amount of water (129 ± 10.5 ml), which was much less than that of the diseased and normal medication groups. This implies that the physiological stress or fluid imbalance brought on by CCl_4 toxicity was lessened by stevia pretreatment.

Vol.9, No.06; 2025

ISSN: 2581-3366

Table 7. Effect of Treatment on Water Intake

Total Water (500ml)	Groups	Mean ± SD	df	F	Sig.
Water Intake	Diseased	149 ± 10.8[c]	2	26.119	.000
	Standard Drug	142 ± 9.3[b]			
	Treatment	129 ± 10.5[a]			

The diseased group consumed the most feed (142.8 ± 9.5 g), which may have been a compensatory reaction to metabolic abnormalities brought on by the liver. The Stevia-treated group (140 ± 8.4 g) maintained a consumption that was comparable to both groups, while the normal medication group had a somewhat reduced intake (137.5 ± 9.8 g).

Feed intake differences between groups, however, were not statistically significant ($p = 0.114$), suggesting that CCl_4 toxicity and therapeutic interventions (drug or Stevia) had little effect on total food intake.

Table 8. Effect of Treatment on Feed Intake

Total Feed (200g)	Groups	Mean ± SD	df	F	Sig.
Feed Intake	Diseased	142.8 ± 9.5[b]	2	2.233	.114
	Standard Drug	137.5 ± 9.8[a]			
	Treatment	140 ± 8.4[ab]			

4. Discussion

In the present study, rats were pre-treated with Stevia for 28 days before being challenged with carbon tetrachloride (CCl_4), a known hepatotoxin, at the end of the trial. It helped to assess the phytochemical characterization and hepatoprotective potential of Stevia rebaudiana in an experimental model. There were four groups in the research design including normal, diseased, standard drug, and treatment group. Throughout the 28-day trial, the diseased group was kept on a normal diet and was only exposed to CCl_4 at the end of the trial without any prior protective intervention. The normal control group, on the other hand, received a standard diet without treatment or CCl_4 exposure but underwent pre-testing and post-testing to evaluate any changes over time. The standard drug group got Silymarin daily for 28 days, followed by CCl_4 injection to assess its protective effect. The treatment group was given Stevia extract daily along with a

Vol.9, No.06; 2025

ISSN: 2581-3366

typical diet for 28 days before receiving CCl_4 to investigate its capacity to prevent liver damage. This strategy highlights Stevia's preventive rather than therapeutic potential, and the findings confirm its position as a hepatoprotective drug.

The antioxidant profile of Stevia rebaudiana extract, as assessed by multiple in vitro experiments, revealed a strong capability for scavenging free radicals and reducing oxidative stress, which was deemed critical for its hepatoprotective action. The DPPH assay showed a 61.04% inhibition, showing a high free radical scavenging ability, most likely due to the presence of active phytochemicals such as diterpene glycosides, phenolics, and flavonoids. The total phenolic content (TPC) was determined to be 3265 mg/100 ml GAE, indicating a high concentration of phenolic substances recognized for their capacity to neutralize reactive oxygen species and prevent lipid peroxidation. The extract contains abundant polyphenolic antioxidants, as evidenced by a total flavonoid content (TFC) of 3790.50 µg/ml of quercetin. These chemicals were recognized to boost endogenous antioxidant defenses and shield cell structures from oxidative damage. The extract's ferric reducing antioxidant power (FRAP) of 13.71 mM $FeSO_4$ demonstrates its capacity to donate electrons, which is crucial for stabilizing oxidized molecules and maintaining cellular redox equilibrium. Overall, these findings offered compelling evidence that Stevia rebaudiana is a rich source of natural antioxidants, which most likely contributed to its reported hepatoprotective benefits by lowering oxidative stress in liver tissues. These findings were consistent with prior research that validated and supported stevia's therapeutic promise in treating oxidative liver damage, necessitating additional exploration into its mechanisms of action [17].

Body weight was tracked throughout the course of the 28-day period as a measure of overall health. Even though all groups gained weight to some extent, the diseased group gained the least, which may indicate that the CCl_4 dosage had immediate hepatotoxic effects that hampered development and metabolic efficiency. On the other hand, a healthy increase in body weight was seen in both the Stevia-treated and standard drug group, which was comparable to the normal control. This suggests that either silymarin or stevia pretreatment reduced the harmful effects of CCl_4, maybe by preserving liver metabolic function and boosting physiological resistance. Stevia's potential as a natural substitute for common hepatoprotective medications is shown by the fact that rats fed with it displayed weight trends that were similar to those of the silymarin group. In a similar vein, earlier studies also noted that treated rats showed better development patterns. Beyond biochemical stability, these results corroborate our findings that stevia improves general health status [18].

All groups' serum glucose levels stayed comparatively constant, with no statistically significant changes seen between baseline and post-treatment readings. The lack of noticeable hyperglycemia or hypoglycemia indicates that the brief exposure to CCl_4 did not significantly impair glucose control, even if it may have an indirect effect on glucose metabolism through liver injury. Furthermore, the group that received Stevia did not show increased glucose levels, confirming that Stevia does not cause hyperglycemia and may potentially help maintain

Vol.9, No.06; 2025

ISSN: 2581-3366

glycemic stability, a characteristic that has been backed by earlier research on its possible antidiabetic benefits. Stevia leaves powder dramatically reduced food intake and serum blood glucose levels in diabetic mice by 38% [19]. These effects, however, are secondary and call for further focused investigation because glucose metabolism was not the major focus of this study and no diabetes model was used.

The lipid profile revealed significant differences across the groups and offered more insight into the preventive benefits of stevia. Serum cholesterol, LDL, triglycerides, and VLDL levels were significantly higher in the diseased group, which was not treated prior to CCl_4 administration. This is consistent with the dyslipidemia often seen in hepatotoxic situations. This is to be expected as hepatic damage causes fat buildup in the plasma by impairing lipid metabolism. In spite of the CCl_4 challenge that followed, the 28-day Stevia pre-treatment successfully maintained hepatic lipid-processing function, as seen by the much lower levels of these lipids in the Stevia-treated group as compared to the diseased group. The standard drug group likewise exhibited this protective lipid-lowering effect, indicating that silymarin and stevia are equally effective. It's interesting to note that HDL levels were marginally higher in the diseased group, most likely as a result of changed lipoprotein dynamics after liver damage or a compensatory reaction to oxidative stress. HDL levels were adjusted in the silymarin and stevia groups, which further suggests that lipid transport and balance had been restored. In line with its antioxidant mediated membrane stabilizing capabilities, the total lipid content showed a similar pattern, with a protective downward trend in the Stevia group and a substantial rise in the diseased group. These results are in line with other study that showed that Stevia has lipid lowering effects in models of metabolic and hepatic disorders [20]. This supports stevia's function in maintaining metabolic balance and protecting the liver, which is consistent with a thorough lipid profile investigation.

Liver function tests offered compelling biochemical proof of hepatoprotection. After being exposed to CCl_4, the diseased group's levels of ALT, AST, and ALP, three important indicators of hepatocellular integrity rose considerably, indicating membrane rupture and enzyme leaking into the blood. These increases were significantly reduced in both the Stevia and regular medication groups, which clearly implies that the 28-day pretreatment strengthened hepatic cellular membranes and stopped CCl_4's harmful effects. Particularly, the treatment group displayed enzyme levels that were close to the normal control, highlighting its strong cytoprotective and antioxidant properties. The idea that phytoconstituents such as flavonoids, phenolics, and glycosides assist hepatic defense mechanisms by neutralizing free radicals and boosting endogenous antioxidant systems is supported by the decrease in these enzymes after Stevia pre-treatment. On the other hand, earlier research on hepatic inflammation revealed encouraging similarities. According to the investigations, stevia hydroalcoholic extract reduced LPS-induced liver damage by lowering proinflammatory cytokines TNF α, IL 1β, and IL 6 and restoring normal ALT/AST levels [21]. This supports our understanding of the mechanism by which stevia's flavonoid rich extract reduces oxidative and inflammatory liver damage by showing that it consistently reduces inflammation in response to various hepatic stressors.

Vol.9, No.06; 2025

ISSN: 2581-3366

Liver function was also reflected in protein metabolism. Albumin levels were somewhat lower in the diseased group, which is explained by decreased hepatic protein synthesis after exposure to CCl_4. Stevia and silymarin pre-treatment successfully preserved steady protein metabolism, as shown in the normal group, although they did not considerably raise albumin levels over the usual range. The diseased group had higher total protein levels, which may have been brought on by an inflammatory reaction and increased globulin production. Total protein levels returned to normal after stevia pretreatment, indicating that inflammation had subsided and the hepatic synthetic balance had been restored.

Serum bilirubin, another crucial indicator of liver function, was much higher in the diseased group, suggesting compromised hepatobiliary excretion. Stevia's cholagogue and hepatoprotective properties were supported by the group who received it, as seen by a notable drop in bilirubin levels that was nearly identical to the normal control. This bilirubin lowering impact suggests that stevia promotes the liver's excretory processes and shields hepatocytes from harm, maybe by preserving bile flow and averting cholestasis. In earlier studies, stevia was shown to dramatically lower bilirubin levels in rats with CCl4-induced liver cirrhosis. This is probably because stevia has anti-inflammatory and antioxidant qualities that help shield the liver from harm [16].

To ascertain the systemic effect of CCl_4 and the protective range of stevia, renal function was evaluated in addition to hepatic indicators. The diseased group's serum creatinine, a crucial measure of renal filtration capacity, was much higher, indicating that liver damage may have indirectly harmed kidney function. Stevia pretreatment significantly decreased creatinine levels, even more than the standard drug group, suggesting that it may have nephroprotective as well as hepatoprotective effects. Under research settings, there was no disturbance to nitrogen metabolism, as seen by the relatively constant serum urea levels across groups. This renal protection highlights the systemic antioxidant capacity of stevia, even if it is not the main emphasis. One of the previously done researches also presented reduced creatinine levels, suggesting nephroprotection, followed by Stevia residue extract attenuated renal injury in CKD mice by suppressing TGF-β1 and improving oxidative or inflammatory indicators [22].

Indicators of behavior and metabolism, such as feed and water consumption, provide more information on the health of the animals. Polydipsia, a common reaction to systemic toxicity and metabolic stress, was seen in the ill group. Following the CCl_4 assault, the Stevia-treated group's water intake was considerably lower, indicating better systemic homeostasis and less stress. All groups' feed intake was rather constant, indicating that neither toxicity nor therapy had a substantial impact on hunger and that the outcomes were actually the result of biochemical protection rather than variations in food intake. Contrary to our results, other earlier research also shown that groups treated with stevia consumed more water than the control group. In a similar vein, those treated with Stevia also had a lower feed efficiency ratio, suggesting possible impacts on eating behavior [23].

Vol.9, No.06; 2025

ISSN: 2581-3366

Overall, this study offers compelling proof that when taken for an extended length of time before a toxic assault, Stevia rebaudiana has notable prophylactic hepatoprotective action. Stevia's capacity to prepare the liver against oxidative and inflammatory damage is supported by the normalization of liver enzymes, lipid profile, bilirubin, and creatinine levels in the treated group relative to the sick group. Stevia's rich phytochemical makeup, which includes flavonoids, terpenoids, and polyphenols that strengthen antioxidant defenses, maintain cellular membranes, and promote metabolic function, is probably what mediates these beneficial actions. Notably, Stevia's hepatoprotective effectiveness was on par with that of silymarin, a well-known hepatoprotective drug, indicating its promise as a plant-based, natural option for managing liver health. In addition to validating the traditional applications of stevia in herbal medicine, these findings are consistent with earlier research and pave the way for the development of stevia into functional foods and nutraceuticals that prevent liver disorders. Isolating certain bioactive chemicals, clarifying their modes of action, and assessing their long-term safety and effectiveness in various disease models should be the main goals of future research.

5. Conclusion

The present study effectively illustrated Stevia rebaudiana's phytochemical diversity and strong hepatoprotective effects in a rat liver toxicity experimental model generated by carbon tetrachloride (CCl_4). High quantities of phenolics and flavonoids were found in stevia extract, according to phytochemical research. These compounds greatly enhanced the extract's antioxidant activity, as demonstrated by its high total phenolic and flavonoid content, powerful DPPH radical scavenging, and ferric reduction capacity (FRAP). These in vitro findings validated the idea that stevia has significant anti-free radical and anti-oxidative stress properties.
Stevia's hepatoprotective properties were further confirmed by in vivo tests. Stevia pretreatment for 28 days significantly protected rats against CCl_4-induced hepatic damage, as seen by reduced blood creatinine concentrations, better lipid profiles, stable protein and bilirubin levels, and normalized liver enzyme levels (ALT, AST, and ALP). The effectiveness of stevia as a natural preventive agent was highlighted by the fact that the group treated with it showed similar biochemical and physiological results to those shown in the group treated with silymarin, the conventional medication. Stevia's systemic protective impact was further supported by the fact that it helped preserve general health markers including body weight, water intake, and feed consumption.

These results collectively suggest that Stevia rebaudiana has hepatoprotective benefits via a variety of pathways, chiefly by boosting antioxidant defenses, preserving membrane integrity, regulating lipid metabolism, and promoting hepatic and renal function. The study demonstrates stevia's potential as a safe, natural substitute for existing hepatoprotective medications and offers a scientific justification for its traditional usage in liver-related conditions. To identify the precise bioactive substances causing these effects and investigate their potential therapeutic uses in chronic liver illnesses and more general toxicological models, more investigation is necessary.

Vol.9, No.06; 2025

ISSN: 2581-3366

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Vol.9, No.06; 2025

ISSN: 2581-3366

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