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RESEARCH ARTICLE

Enzymatic and Non-enzymatic Antioxidants Elevation by Polyphenol Rich Extract Derived from *Stevia rebaudiana*, *Musa acuminata* and *Solanum lycopersicum*

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Abstract

Background: Oxidative stress arises due to a misbalance between radical formation and detoxification. This results in micro-macromolecular damage and redox signaling disruption. An antioxidant hinders the oxidation of the substrate when it is present at concentrations even lower than those of an oxidizable substrate. **Aim:** Aqueous extract derived from Stevia leaves, banana fruits and tomato fruits screened in healthy and oxidatively stressed Albino Wistar rats (10-12 weeks old) for developing a nutraceutical or therapeutic option for disorders involving free radicals. **Methods:** Aqueous extract subjected to oral OD dose of 100 mg/kg for 30 days using ascorbic acid as standard. Body weight, enzymatic antioxidant defenses (superoxide dismutase, glutathione reductase and catalase) and non-enzymatic antioxidants (ascorbic acid, alpha-tocopherol) were determined. **Results:** Oral extract administration increased enzymatic and non-enzymatic antioxidant levels. Compared to Stevia and control groups, the extract significantly controlled body weight gain. Higher non-enzymatic antioxidant levels indicated the presence of water-soluble antioxidants. H and E- stained organ sections confirmed improved physiology in extract-treated groups. **Conclusion:** The combined aqueous extracts of stevia, ripe or unripe banana and ripe or unripe tomato are an appropriate strategy for maintaining normal oxidative level and reducing free radicals. This paves the path for further phytochemical and pharmacological screening in disorders like metabolic syndrome.

Major Findings: The polyphenol-rich extract from *Stevia rebaudiana*, *Musa acuminata*, and *Solanum lycopersicum* enhanced both enzymatic and non-enzymatic antioxidants in rats. Histology confirmed improved organ health, and body weight gain was regulated. The extract showed synergistic effects and potential against oxidative stress.

Keywords: Antioxidant, Ascorbic Acid, Catalase, Glutathione Reductase, Stevia, Superoxide Dismutase

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1. Introduction

Disruption between radical formation and detoxification forces the physiological system towards oxidative stress and impairs redox-sensitive target proteins[1,2]. This inconsistency between pro-oxidative and anti-oxidative pathways may result in disorders like atherosclerosis and diabetes[3]. An antioxidant restores the equilibrium by retarding or inhibiting the oxidation of the substrate even at concentrations smaller than of oxidizable substrate. Superoxide dismutase and catalase provide enzymatic antioxidant defenses, while ascorbic acid, vitamins represent the non-enzymatic antioxidants[4]. High calorie intake is also associated with metabolic disorders, and reformulation of pharmaceutical products may be an acceptable means of minimizing sugar consumption by partial or complete replacement of sugar[5]. Polyphenols are strong antioxidants and beneficial in disorders such as diabetes, obesity and neurodegenerative diseases[6]. *S. rebaudiana* leaves are been employed for sucrose substitution and steviol glycosides ensure low calories with high levels of sweetness[7]. Polyphenols present in *M. acuminata* and *S. lycopersicum* fruit have an edge because their assimilation is increased by saccharides[8,9].

In a previous study, our research group fortified the aqueous extract of *S. rebaudiana* leaves with fruit extracts of *M. acuminate* and *S. lycopersicum*[9]. Ripe and unripe fruit aqueous extracts were mixed in various ratios and screened for DPPH inhibition. The dual component fruit extracts possessing the highest free radical scavenging were further intermingled with stevia aqueous extract[10]. The obtained triple component extract (pH 4.6) consisted of aqueous extracts of Stevia leaves: Ripe banana fruit: Unripe banana fruit: Ripe tomato fruit: Unripe tomato fruit in a ratio of 7:2:1:1:2. The extract exhibited significant total phenolic content, total flavonoid content and DPPH inhibition activity. The extract was screened for acute toxicity in Albino Wistar rats as per OECD 423 guidelines. That study ensured that the oral administration of the extract is non-toxic and LD_{50} cut off to be 2500 mg/kg of rat body weight. The screening drilled the prospective pharmacological screening of the extract at a dose of 40-200 mg/kg rat body weight.

Stevia enjoys diverse biological activities like antioxidant and banana's dopamine, ascorbic acid and other antioxidants reduce oxidative stress[11,12]. Pronounced amount of vitamin C, folate and vitamin K reinforces *S. lycopersicum* to act as a powerful antioxidant[13,14]. Two or more active ingredients may be added in a dosage form and fortification of bio actives[15,16]. We assessed its antioxidant potential so that it may be further utilized in free radical-mediated disorders such as metabolic syndrome.

2. Materials and Methods

2.1 Chemicals

Thiobarbituric acid, trichloroacetic acid, n-butanol, 1,1,3,3-tetraethoxypropane, H_2O, pyrogallol, Tris HCl, 5, 5'-dithiobis-2-nitrobenzoic acid, Folin ciocalteau reagent, Bovine serum albumin, copper sulfate, sodium-potassium tartrate were purchased from Sigma Aldrich (India).

2.2 Plant Materials

Stevia rebaudiana Bertoni. leaves (family: *Asteraceae*), *M. acuminata* Colla. fruits (family: *Musaceae*) and *S. lycopersicum* L. fruits (family: *Solanaceae*) were purchased from the local market and subjected to authentication (IU/PHAR/HRB/20/03, IU/PHAR/HRB/20/01, IU/PHAR/HRB/20/02) at Department of Pharmacy, Integral University, Lucknow, India. The triple component extract consisted of aqueous extracts of Stevia leaves: Ripe banana fruit: Unripe banana fruit: Ripe tomato fruit: Unripe tomato fruit in a ratio of 7:2:1:1:2.

2.3 Animal species

Albino Wistar rats of 8-12 weeks age were procured from Central Drug Research Institute, Lucknow, India (Reg. No. 34/GO/Re-SL/Bi-S/99/CPCSEA) and studied at Hygia Institute of Pharmaceutical Education and Research, Lucknow, India (Reg. No. 1088/Re/S/07/CPCSEA) as per the approved protocol: HIPER/IAEC/43/20/08[17].

2.4 Statistical Analysis

Statistical difference between groups was measured using GraphPad Prism 8.4.3.686. Values are depicted as mean ± SEM for 3 observations per experiment/

group. Statistical difference between experiment/ groups was measured using non-parametric paired t-test, where variations between groups are reported as non-significant ($p > 0.05$), significant ($p \leq 0.05$), very significant ($p \leq 0.01$), highly significant ($p \leq 0.001$) when the test is compared to the reference or control.

3. Antioxidant Screening

3.1 Control and Standard Group

25 rats were divided into 5 groups (I-V) comprising of 5 rats in each. Group I and II demonstrated the study for healthy physiology. Groups III, IV and V exhibited the oxidative stress and were administrated intraperitoneal injection of a single dose of CCl_4 (50% in corn oil) at 0.5 ml/kg body weight at day 0[18]. For 30 days: Group I (Normal control) received vehicle (0.9% normal saline) only. Group II (Test in normal physiology) received vehicle + triple component extract (10) (100 mg/kg, p.o., OD). Group III (Disease control) received vehicle only[19]. Group IV (Standard) received vehicle + Ascorbic acid (100 mg/kg, p.o., OD). Group V (Test in oxidative stress) received vehicle + triple component extract (100 mg/kg, p.o., OD). Body weight (Table 1) and food intake were monitored daily and estimations carried out for the antioxidant activity in serum (Vitamin C and E) on day 0, 5, 10, 15, 20, 25 and 30 of the study by taking blood samples from retro-orbital sinus using fine capillary tubes[20,21]. Antioxidant profile of liver, brain, kidney and spleen including histopathological studies was accomplished.

3.2 Rat Organ Homogenate (10% w/v)

Rat's organs were removed, perfused, and homogenized in 50 mM/L phosphate buffer (pH 7.4), containing 120 mM KCl (ratio 1:10 w/v). The supernatant was collected after centrifuging the homogenate at 3000 rpm for 10 minutesat 4° C[22].

3.3 Thiobarbituric Acid Reactive Substances

The secondary degradation product of lipid oxidation (malonaldehyde), reacts with thiobarbituric acid. These 2 on heating in acidic solutions forms a pink colored schiff's base adduct (absorbs at 532-535 nm)[16]. The decrease in absorbance of the sample as compared to the control evaluates the protection against lipidperoxidation.

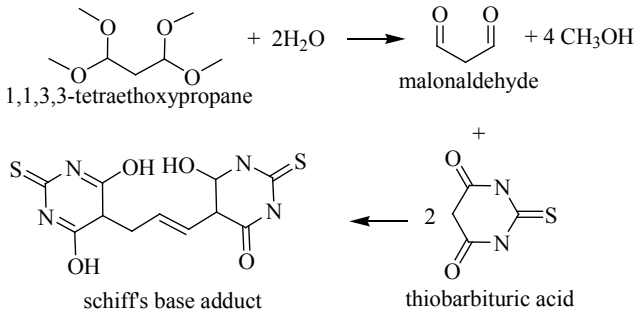


Figure 1. Reaction involved in thiobarbituric acid reactive substances assay.

TBARS were extracted in trichloroacetic acid, and the absorbance of the pink organic phase was evaluated at 532 nm[23,24]. The levels of lipidperoxides (TBARS) expressed as nmoles of thiobarbituric acid reactive substances/mg protein using a standard curve of 1,1,3,3-tetraethoxypropane (hydrolyzes in acid to form malonaldehyde)[25-27].

3.4 Catalase

Catalase activity was determined as per Aebi and calculated as η moles of H_2O_2 consumed/minute/mg protein[28]. Catalase = [ΔA/minute × volume of assay] / [0.081 × volume of homogenate × mg of protein].

3.5 Superoxide Dismutase (SOD)

Superoxide dismutase was determined based on inhibition of the autoxidation of pyrogallol[29].

3.6 Tissue Glutathione

Organ tissue (500 mg) was homogenized in 5 ml of 0.02 M EDTA and added 4.0 ml of cold distilled water. After mixing it well, 1 ml of 50 % trichloroacetic acid was added and shaken intermittently for 10 minutes using a vortex mixer. After 10 minutes, the contents were centrifuged at 6000 rpm for 15 minutes. Following centrifugation, 2 ml of the supernatant was mixed with 4.0 ml of 0.4 M Tris buffer (pH 8.9) and 0.1 ml of 0.01M DTNB. The absorbance was read within 5 minutes of the addition of DTNB at 412 nm against a reagent blank.

3.7 Protein Estimation

Protein reacts with Folin ciocalteau reagent to give a colored complex and the absorbance determined at 750 nm. The reaction involves reduction of copper and Folin reagent by the tyrosine, tryptophan and cysteine present in the protein. Calibration curve for protein

was prepared by using bovine serum albumin solution (2 mg/ml).

3.8 Histology

Stained slides of liver, brain, kidney and spleen were visualized by using a light microscope[30].

4. Result

Oral extract administration increased enzymatic and non-enzymatic antioxidant levels. Compared to Stevia and control groups, the extract significantly controlled body weight gain. Higher non-enzymatic antioxidant levels indicated the presence of water-soluble antioxidants. H- and E - stained organ sections confirmed improved physiology in extract-treated groups.

5. Discussion

Normal physiological conditions ensure free radical scavenging by natural cellular defense, comprising enzymes like superoxide dismutase and catalase[35]. Catalase converts H_2O_2 catalytically into water and oxygen, and thus neutralizes it[36]. Superoxide is dismutated to less toxic compounds by superoxide dismutase[37].

Table 1. Body weight of rats (g) at dose of 100 mg/kg

	Normal		Oxidative Stress		
Observation Time	**Control**	**Test**	**Control**	**Standard**	**Test**
Day 0	198.52 ± 2.51	202.03 ± 0.48*	197.15 ± 2.76	200.90 ± 1.16*	198.80 ± 2.38*
Day 1	201.65 ± 3.11	203.12 ± 0.57*	199.24 ± 2.12	202.26 ± 1.31*	201.23 ± 2.66*
Day 2	203.86 ± 3.06	204.80 ± 0.92*	201.25 ± 2.61	202.60 ± 0.97*	203.77 ± 2.60*
Day 3	205.87 ± 1.45	206.41 ± 1.33*	203.04 ± 1.89	204.13 ± 0.94*	205.25 ± 2.58*
Day 4	206.98 ± 1.26	208.50 ± 1.08*	204.30 ± 2.78	206.43 ± 0.73*	207.28 ± 2.72*
Day 5	208.53 ± 3.48	209.95 ± 0.94*	205.65 ± 1.88	208.68 ± 2.91*	208.50 ± 0.82*
Day 6	209.95 ± 3.40	212.31 ± 0.73*	207.27 ± 2.93	210.80 ± 0.95*	209.85 ± 2.69*
Day 7	211.24 ± 1.34	213.43 ± 1.01*	208.56 ± 2.87	212.78 ± 0.64*	211.61 ± 2.85*
Day 8	213.06 ± 1.42	215.01 ± 1.45*	209.58 ± 2.41	215.33 ± 0.91*	212.96 ± 2.72*
Day 9	214.35 ± 3.37	216.63 ± 1.93*	211.00 ± 2.43	217.59 ± 1.95*	214.32 ± 2.62*
Day 10	215.47 ± 2.88	218.55 ± 2.12*	211.98 ± 1.93	220.57 ± 1.96*	216.01 ± 2.32*
Day 11	217.09 ± 2.88	220.81 ± 1.85*	213.20 ± 1.62	222.63 ± 0.98*	217.06 ± 2.08*
Day 12	218.41 ± 2.73	222.83 ± 1.43*	214.39 ± 1.33	223.80 ± 1.13*	218.01 ± 1.69*
Day 13	219.13 ± 2.51	224.65 ± 2.11*	215.14 ± 1.07	224.66 ± 0.96*	218.87 ± 1.48*
Day 14	219.78 ± 1.56	226.31 ± 1.27*	215.75 ± 1.09	226.18 ± 1.61*	219.40 ± 2.21*
Day 15	220.48 ± 2.43	228.28 ± 1.16*	216.46 ± 0.94	227.40 ± 0.92*	220.14 ± 2.33*
Day 16	221.16 ± 2.36	230.17 ± 1.13*	217.15 ± 0.84	228.62 ± 0.95*	220.82 ± 1.23*
Day 17	221.85 ± 1.28	232.06 ± 2.10*	217.50 ± 0.47	228.24 ± 2.91*	221.51 ± 1.12*
Day 18	222.54 ± 2.21	233.94 ± 1.08*	218.19 ± 0.35	231.61 ± 1.56*	222.20 ± 1.02*
Day 19	223.22 ± 2.15	235.83 ± 2.05*	218.21 ± 0.56	232.95 ± 1.54*	222.88 ± 0.92*
Day 20	223.91 ± 2.09	237.72 ± 1.03*	218.90 ± 0.49	234.20 ± 1.53*	223.57 ± 0.82*
Day 21	224.60 ± 2.03	239.60 ± 1.00*	219.58 ± 0.43	235.12 ± 1.60*	224.26 ± 0.73*
Day 22	225.62 ± 1.80	241.49 ± 0.98*	220.27 ± 1.41	236.38 ± 2.59*	224.94 ± 0.65*
Day 23	226.30 ± 1.74	243.38 ± 0.95*	220.96 ± 0.41	237.96 ± 2.52*	225.63 ± 0.58*
Day 24	226.99 ± 1.69	245.26 ± 0.93*	221.64 ± 1.45	239.15 ± 1.03*	226.32 ± 0.53*
Day 25	228.01 ± 1.46	247.15 ± 1.91*	222.33 ± 0.51	240.21 ± 1.55*	227.00 ± 1.31*
Day 26	228.70 ± 1.40	249.04 ± 0.89*	223.02 ± 0.59	241.70 ± 1.22*	227.69 ± 1.50*
Day 27	230.05 ± 1.11	250.92 ± 0.87*	223.70 ± 0.68	243.26 ± 0.56*	228.38 ± 0.52*
Day 28	230.74 ± 1.02	252.81 ± 1.84*	224.43 ± 2.78	244.81 ± 1.82*	229.62 ± 0.59*
Day 29	232.09 ± 1.00	254.56 ± 0.81*	225.31 ± 2.90	246.36 ± 1.81*	230.70 ± 2.57*
Day 30	233.20 ± 0.87	255.28 ± 0.92*	225.52 ± 0.92	248.46 ± 0.56*	232.12 ± 0.99*

Values are depicted as mean ± SEM for five rats per group.

Table 2. Antioxidant status at dose of 100 mg/kg

Observation Time	Normal		Oxidative Stress		
	Control	Test	Control	Standard	Test
Superoxide Dismutase (units/mg of protein)[31]					
Liver	82.5901± 2.56	86.2594 ± 2.06**	66.3245 ± 1.94	78.3216 ± 2.45***	72.0361 ± 2.42**
Brain	21.7425 ± 0.64	23.0364 ± 1.03**	14.0259 ± 1.26	19.0852 ± 2.13***	17.8216 ± 1.94**
Spleen	28.0269 ± 1.06	31.3015 ± 1.57**	21.6201 ± 1.69	24.3069 ± 0.94***	22.2018 ± 0.46**
Kidney	35.3204 ± 3.61	38.8520 ± 3.18**	28.7530 ± 2.67	32.4025 ± 2.14***	29.7009 ± 1.94**
Catalase (units/mg of protein)[31]					
Liver	51.4360 ± 1.65	55.7689 ± 1.42**	39.7415 ± 1.68	45.7613 ± 2.06***	43.7469 ± 1.08**
Brain	27.0258± 2.16	32.0256 ± 2.03**	22.4109 ± 2.10	26.8026 ± 2.36***	24.1496 ± 2.07**
Spleen	32.0146 ± 4.06	35.7456 ± 3.84**	26.1364 ± 2.46	29.9761 ± 1.37***	27.9426 ± 1.43**
Kidney	56.7429 ± 4.26	61.1569 ± 3.43**	47.8016 ± 3.10	53.7029 ± 2.94***	49.7530 ± 2.10**
Thiobarbituric Acidreactive Substances (nmoles MDA/g wet weight)[25]					
Liver	246.0358 ± 10.56	229.4125 ± 15.46**	335.7436 ± 12.67	291.0258 ± 19.63***	321.2480 ± 17.56**
Brain	206.1578 ± 8.56	188.7532 ± 6.08**	246.7128 ± 11.20	214.3546 ± 15.38***	234.3215 ± 9.41**
Spleen	131.3671 ± 5.34	114.2689 ± 5.46**	185.3645 ± 9.43	158.0258 ± 9.43***	177.3105 ± 10.73**
Kidney	188.0389 ± 3.09	163.0248 ± 7.06**	236.5980 ± 13.05	207.9615 ± 9.13***	229.9732 ± 13.47**
Tissue Glutathione GSH (µM / mg of protein)[32]					
Liver	37.0315 ± 3.26	46.7410 ± 3.49**	25.8425 ± 2.46	48.6189 ± 2.13***	43.7159 ± 1.94**
Brain	22.3658 ± 1.39	29.8506 ± 3.16**	16.6309 ± 3.16	34.3043 ± 2.16***	27.7025 ± 2.17**
Spleen	26.6015 ± 1.46	39.4305 ± 2.48**	19.7402 ± 2.46	41.4028 ± 2.09***	36.6018 ± 2.06**
Kidney	29.6320 ± 2.40	37.9025 ± 3.19**	22.6008 ± 2.73	42.0368 ± 1.16***	35.8467 ± 2.17**
Vitamin C[33] (mgm/g wet tissue)					
Liver	0.2546 ± 0.06	0.2737 ± 0.03**	0.1960 ± 0.07	0.2309 ± 0.01***	0.2169 ± 0.04**
Brain	0.2159 ± 0.04	0.2467 ± 0.04**	0.1671 ± 0.06	0.1970 ± 0.05***	0.1762 ± 0.06**
Spleen	0.1827 ± 0.03	0.1957 ± 0.02**	0.1409 ± 0.04	0.1664 ± 0.06***	0.1491 ± 0.01**
Kidney	0.1694 ± 0.02	0.1806 ± 0.04**	0.1337 ± 0.05	0.1503 ± 0.03***	0.1409 ± 0.03**
Vitamin E (µmol / mg tissue)[34]					
Liver	0.8615 ± 0.01	0.9190 ± 0.07**	0.5743 ± 0.08	0.7671 ± 0.01***	0.6903 ± 0.04**
Brain	0.4931 ± 0.07	0.5479 ± 0.03**	0.3796 ± 0.04	0.4760 ± 0.06***	0.4538 ± 0.13**
Spleen	0.5643 ± 0.06	0.5970 ± 0.04**	0.4289 ± 0.03	0.5367 ± 0.07***	0.4830 ± 0.01**
Kidney	0.5736 ± 0.08	0.5864 ± 0.06**	0.4367 ± 0.09	0.5438 ± 0.03***	0.4964 ± 0.06**

Values are depicted as mean ± SEM for five rats per group.

Table 3. Vitamin C(mgm/mL of serum) at dose of 100 mg/kg

Observation Time	Normal		Oxidative Stress		
	Control	Test	Control	Standard	Test
Day of dosing	0.3459 ± 0.01	0.3521 ± 0.02**	0.2194 ± 0.06	0.2153± 0.01***	0.2208± 0.03**
Day 5	0.3522 ± 0.04	0.3553 ± 0.04**	0.2273 ± 0.04	0.2276± 0.04***	0.2286± 0.04**
Day 10	0.3370 ± 0.06	0.3606 ± 0.08**	0.2473 ± 0.09	0.2469± 0.06***	0.2371± 0.06**
Day 15	0.3419 ± 0.07	0.3661 ± 0.03**	0.2308 ± 0.08	0.2780± 0.07***	0.2497± 0.01**
Day 20	0.3402 ± 0.03	0.3680 ± 0.01**	0.2237 ± 0.04	0.2961± 0.03***	0.2668± 0.08**
Day 25	0.3467 ± 0.01	0.3818 ± 0.07**	0.2460 ± 0.03	0.3078± 0.04***	0.2784± 0.03**
Day 30	0.3394 ± 0.06	0.4068 ± 0.03**	0.2473± 0.04	0.3261± 0.05***	0.2963± 0.02**

Values are depicted as mean ± SEM for five rats per group.

Table 4. Vitamin E (µmol / mL of serum) at dose of 100 mg/kg

Observation Time	Normal		Oxidative Stress		
	Control	Test	Control	Standard	Test
Day of dosing	0.9232 ± 0.03	0.9231± 0.06**	0.4267 ± 0.04	0.4319 ± 0.06***	0.4271± 0.04**
Day 5	0.9248± 0.05	0.9276 ± 0.02**	0.4309 ± 0.02	0.4467± 0.07***	0.4293± 0.07**
Day 10	0.9343± 0.07	0.9361 ± 0.05**	0.4387 ± 0.03	0.4582± 0.04***	0.4378± 0.03**
Day 15	0.9276± 0.04	0.9308 ± 0.07**	0.4218 ± 0.07	0.4674± 0.05***	0.4405± 0.06**
Day 20	0.9381± 0.06	0.9364 ± 0.03**	0.4183 ± 0.04	0.4706± 0.03***	0.4436± 0.09**
Day 25	0.9476± 0.01	0.9421 ± 0.01**	0.4286 ± 0.03	0.4843± 0.09***	0.4518± 0.04**
Day 30	09490± 0.08	0.9536 ± 0.08**	0.4308± 0.08	0.4943± 0.07***	0.4324± 0.08**

Values depicted as mean ± SEM for 5 rats per group.

Table 5. H and E stained (40×) microscopic examination of the rat organs

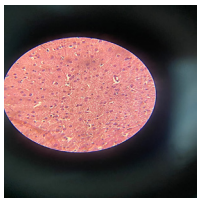
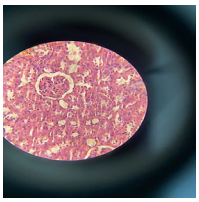
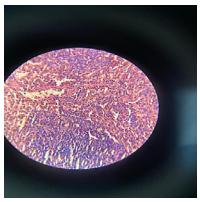
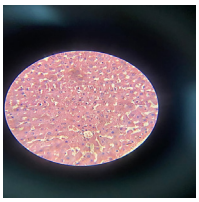
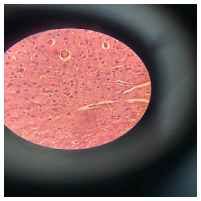
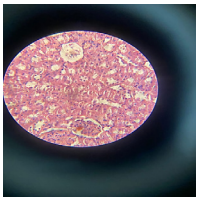
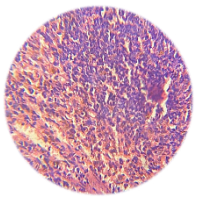
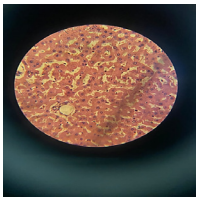
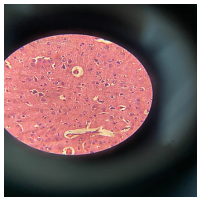
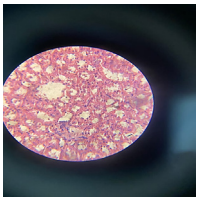
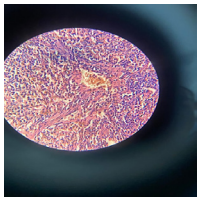
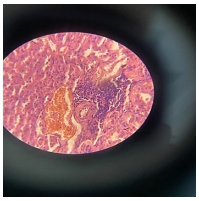
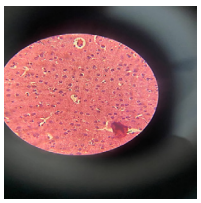
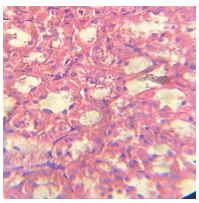
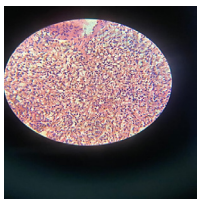
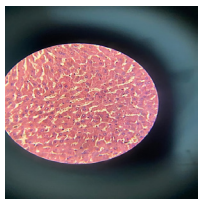
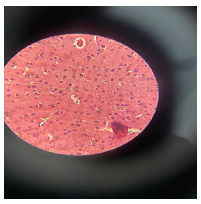
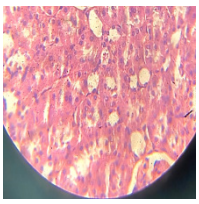
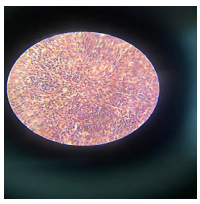
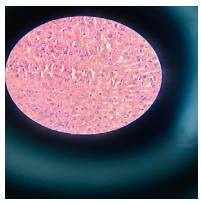
	Brain	Kidney	Spleen	Liver
Normal control				
Test				
Disease Control				

Table 5. Continued...

	Brain	Kidney	Spleen	Liver
Disease standard				
Test				

Hydrogen peroxide is reduced by reduced glutathione in the reaction catalyzed by glutathione peroxidase[38]. Vitamin C (Table 3) supports the regeneration of GSSG back into reduced glutathione[39]. Vitamin E (Table 4) donates hydrogen to scavenge lipid peroxides and terminates oxidative chain reactions. The unoxidised form of vitamin E can be recycled back by vitamin C and glutathione[40].

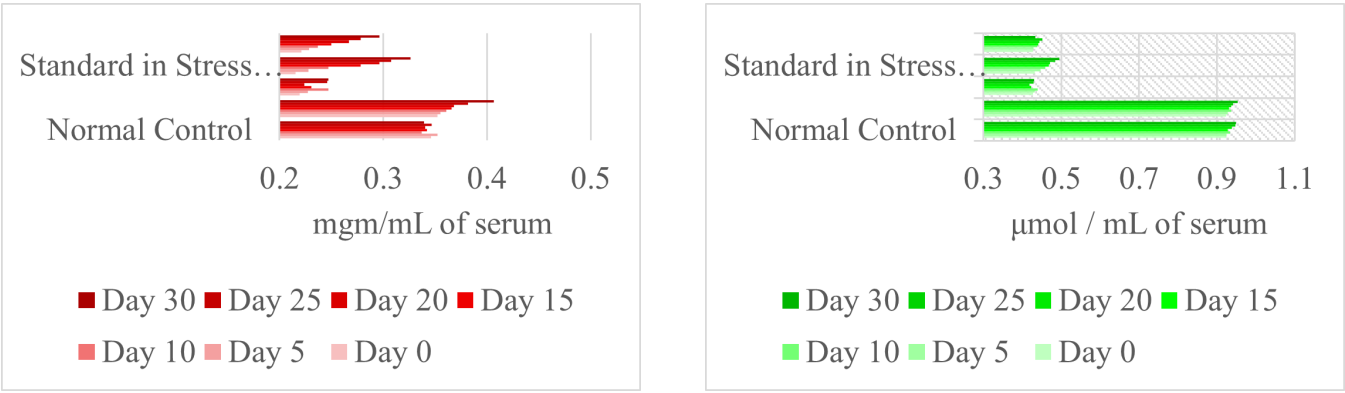
CCl_4 is a potent hepatotoxic agent used for the screening of the antioxidant profile (Table 2) of plant extracts, Oxidative stress involves the reduction in these antioxidant resistance mechanisms along with malondialdehyde formation due to lipid peroxidation[41-43]. The antioxidant activity should be based on at least 2 different test systems and we analyzed the extract for catalase, superoxide dismutase and thiobarbituric acid reactive substances and non-enzymatic antioxidants[44].

The intermingling of water-soluble secondary metabolites of *S. rebaudiana* Bertoni. leaves, *M. acuminata* Colla. fruits and *S. lycopersicum* L. fruits results in significantly enhanced antioxidant activity as compared to control, stevia and fruit extract groups. Our results endorse Gulcin that interactions between constituent's results in effects which are not the obligatory properties of the individual constituents[45]. The activity of the extract may be due to simultaneous solubility of a group of substances and the multiple targets[46]. The differences in substitution influences the phenoxyl radical stability and there by the antioxidant properties of the flavonoids[47]. Pharmacodynamics and pharmacokinetic interactions may have led to synergism[48,49].

The antioxidant activity may be attributed to the antioxidant enzymes of *S. rebaudiana* Bertoni leaves, well known to reduce the risk of oxidative stress[32,50]. Tomato must have contributed to the antioxidant activity as the antioxidant activity is known to be independent of lycopene[51,52]. The results are in coordination with Vijay Kumar that banana decreases malondialdehyde, hydroperoxides and enhances catalase and superoxide dismutase[53,54]. Tricomponent extract administration resulted in a substantial increase in the concentrations of enzymatic and nonenzymatic antioxidants (Figure 1). The presence of water-soluble antioxidants was demonstrated by higher non-enzymatic oxidant levels. Low sugar load of the tricomponent extract ensured significant check on body weight gain (Figure 2). Hematoxylin and eosin-stained sections of organs and hematological parameters indicated an enhanced physiology in the tricomponent extract-treated groups (Figure 3). The cell architecture of the liver in normal mice showed normal hepatocytes with central veins. Significant difference was observed in the liver's physiological status between the tricomponent extract-treated group and the control group[55].

Microscopic screening (Table 5) of spleen, liver, kidney and brain confirmed the enhanced structural modification (Figure3). Hematoxylin and eosin-stained sections showed enhanced histology of brain showing

Figure 2. Enzymatic and non-enzymatic antioxidant status in rat organs during the study at dose of 100 mg/kg.



Vitamin E Vitamin C

Figure 3. Non-enzymatic antioxidant status in rat serum during the study at dose of 100 mg/kg.

grey and white matter of cerebral cortex, enhanced red and white pulp of the spleen. Enhanced histology of liver revealed through the central vein and hepatic lobules along with portal triad. The cortical part of the kidney showed enhanced appearing glomeruli along with the cross section of the proximal and distal arm of collecting tubules. Absence of abnormality was found a similar safety profile for current tomato formulation as reported previously by Galle and Phachonpai[56,57]. The outcomes of the current study have a same opinion as Saad that stevia products are safe and don't cause any health problem[58-61].

6. Conclusion

The intermingling of water-soluble secondary metabolites of *S. rebaudiana* Bertoni. leaves, *M. acuminata* Colla. fruits and *S. lycopersicum* L. fruits result in enhanced antioxidant activity. Oxidative stress is enhanced due to sustained hyperglycemia and its downstream may prevent or reverse the diabetic complication. Stevioside significantly decreases the levels of lipid peroxidation and nitric oxide in the liver and kidney. Plant-derived water-soluble compounds demonstrate a plethora of beneficial biological activities, and this antioxidant extract may be beneficial in free radical-mediated disorders such as diabetes, cardiovascular disorders via its antioxidant mechanism.

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8. Author Contribution

Shom Prakash Kushwaha: Experimental work; Pavan Kumar: Drafting of research findings; Akash Singh: Drafting of research findings; Garima Verma: Drafting of research findings; Smriti Ojha: Drafting of research findings; Syed Misbahul Hasan: Drafting of research findings; Akash Ved: Drafting of research findings; Muhammad Arif: Drafting of research findings; Pushpendra Soni: Drafting of research findings; Sujeet Kumar Gupta: Supervision of work; Neha Mathur: Drafting of research findings; Sushil Kumar: Supervision of work. All authors have read and agreed to the published version of the manuscript.

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