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*Original Research*

# Catechins, theaflavins and ginger freeze-dried extract based functional drink significantly mitigate the hepatic, diabetic and lipid abnormalities in rat model

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**Abstract:** The Current study was planned to explore the therapeutic potential of green tea, black tea and ginger based nutraceuticals (catechins, theaflavins and ginger freeze dried extract) against obesity, diabetes and renal malfunctioning. Bioevaluation study was carried out by involving 250 male Sprague Dawley rats. Accordingly, three types of studies were conducted on the basis of different diets i.e. study I (Hyperglycemic rats), study II (obese rats), study III (liver malfunctional rats) each study comprised of five groups of rats ten in each (Sample size according to power analysis) were provided the five types of drinks *i.e.* control, theaflavin enriched, catechins enriched, ginger extract supplemented and combination of catechins, theaflavins and ginger extract were given to the representative groups. Results showed that the body weight of rats effected significantly with functional drinks in all studies. However, catechin enriched drink (T1) resulted maximum reduction in weight during the entire study. Similarly, T2 exerted maximum decline in cholesterol level during study I, II and III by 11.03 & 10.63, 7.62 & 8.05 and 5.99 & 6.01% whereas LDL by 14.25 & 15.10, 10.45 & 12.10 and 7.25 & 8.01%, respectively (trial 1 & 2). The attenuation in serum glucose and enhancement in insulin level of rats are the indicators for the positive impact of black tea functional drinks. In this context, Catechins+theaflavins+GFD enriched drink (T4) Showed better performance than rest and caused 8.82 & 9.77, 11.03 & 12.23 and 5.83 & 5.96% reduction in glucose. Moreover, the T4 significantly improved the liver and antioxidant enzymes. Accordingly, T4 was proved effective for glutathione enhancement whilst T2 alleviated TBARS efficiently during the investigation. The normal ranges of renal function tests and hematological aspects proved the safety of resultant drinks. From the current exploration, it is concluded that drinks supplemented with theaflavin and catechins & GFD are effectual to mitigate lifestyle related malfunctioning.

***Key words:*** Theaflavins; Catechins, Liver malfunctioning's; Dietary interventions; Oxidative stress.

## Introduction

Oxidative stress is an imbalance between antioxidants and Reactive oxygen species (ROS) and causative factor for various maladies ranges from diabetes to cancer owing to disrupt the body natural hemostasis with special reference to oxidation status (1). Recently, diet-based therapy with special reference to polyphenols has been invigorated worldwide and people are using natural food materials as an intervention against various oxidative stress induced maladies. Among different dietary regimen tools, polyphenolic enriched functional and nutraceutical foods engrossed attention due to their acceptability, easy access, low cost and long administration. In this scenario, green & black tea (*Camellia sinensis*) are the members of *Theaceae* family is examples of plants containing bioactive molecules with unique nutraceutical potential. Likewise, the therapeutic worth of ginger is also well established. Extensive studies have suggested that tea consumption provide numerous health benefits mainly attributed to its polyphenols especially catechins, theaflavin and thearubigins. Green tea is a non-fermented product made from tea leaves and further oxidation of polyphenols is stopped by steaming and drying of these leaves. Green tea is a rich source of catechins about 25% of dry weight basis. In contrary, black tea is fermented tea in which green tea catechins condensed and convert into theaflavin and thearubigins. These theaflavin and thearubigins have strong antioxidant and therapeutic worth (2). Green tea is a promising source of polyphenols dominated by catechins (15-25%), further subdivided into different subgroups like epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) however last present in highest proportion (50%). Conversely, in black theaflavin (3-6%) and thearubigins (12-18%) are the dominated polyphenols that produced by the catechins during fermentation. Theaflavin is a group of four compounds constitute about 3-6% of the black tea polyphenols. Structurally, it consists of hydroxy-substituted benzotropolone ring synthesized by condensation of catechins (3). Being a natural antioxidant, all of these biomolecules radical scavenging and chelating properties owing to the presence of hydroxyl group along with gallic acid moiety. Ginger rhizome (*Zingiber officinale* Roscoe) is particularly used as a spice in the food by the consumers in the world. Ginger blessed with array of phytochemicals denoted it strong pharmacological potential. Among the different phytochemicals shogaols, gingerols and zingiberene considered as most effective. There are consolidated scientific evidences that favor the strong nutraceutical potential

of catechins, theaflavin, thearubigins & gingerol against dyslipidemia, hyperglycemia, renal dysfunntioning and various cancer insurgences due to their strong antioxidative potential. The objective of current investigation was to formulate the nutraceuticals and functional food based dietary intervention. Purposely, catechins & theaflavins were isolated from green and black tea and Ginger freeze-dried (GFD) extract was obtained after freeze drying the ginger antioxidant extract and probed against high cholesterol diet induced obese rats.

## Materials and Methods

Recent research project was carried out in the Functional and Nutraceutical Lab of the Institute of Home & Food Sciences, Government College University, Faisalabad. Green tea and black tea variety (Qi-Men) were procured from the National Tea Research Institute (NTRI), Shinkiari, Mansehra for the isolation of the catechins and the theaflavins, respectively. The locally grown ground ginger was used for recent research project. Further, all the components were mixed together in order to make dietary intervention. The reagents (analytical and HPLC grade) and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

### Dietary intervention preparation

The catechins and theaflavins were isolated from green and black tea, respectively through solvent partition method describe by the Xie *et al.* (4). Whereas, after extraction the ginger extract converted into powder through freeze drying to obtain ginger freeze-dried extract (G.F.D.E). The isolates as well as G.F.D.E were probed for their purity through HPLC and added in the respective drink for the formulation of polyphenol based dietary intervention.

Purposely**,** four kinds of polyphenol-based drink were formulated by incorporating different levels of selected bioactive molecules @ 1000ppm (Table 1). A control deprived of extract was also formulated for comparison. Raw materials used for drink preparation were aspartame, citric acid, CMC, sodium benzoate, food grade color and flavor.

### Bio-evaluation studies

The study program was designed after the review and approval of ethical guidelines set by parent institute which are in compliances with international standards (ERC 2019). For the purpose, 250 Sprague Dawley rats were bordered in the Animal Room of the Department of Pharmacy, Government College University, Faisalabad. The rats were acclimatized by feeding on basal diet for a period of one week. The environmental conditions were control throughout the trial like temperature (23±2ºC) and relative humidity (55±5%) along with 12 hour light-dark period. During efficacy trial, three types of studies were conducted independently by involving sucrose induced diabetic rats & high cholesterol+Sucroose diet fed obese rats & $CCL_4$ diet induced liver malfunctional rats. Each study comprised of five groups of rats ten in each (Sample size according to power analysis). Accordingly, five types of drinks *i.e.* control, theaflavin enriched, catechins enriched, ginger extract supplemented and combination of catechins, theaflavins and ginger extract were prepared considering the stability of the active ingredients and given to the representative groups (Table 02). At the commencement of trial, some rats (total 15 rats and average of results were considered as base line trend) were sacrificed to establish the baseline trend. For the induction of obesity, initially high Cholesterol @ 1.5%, cholic acid @ 0.5% and high sucrose @ 40% were administrated for a period of 14 days. During that tenure Cholesterol, LDL & glucose levels were observed to estimate the onset of obesity Afterwards when values of mentioned tests deviate 50% from normal then the original study was started. Similar approaches were adapted in case of diabtes and liver malfunctioning induction by observing the values of glucose and AST, ALT, ALP, respectively. At the termination of the study, overnight fasted rats were decapitated and blood was collected. For serum collection, blood samples were subjected to centrifugation using centrifuge machine @ 4000 rpm for 6 min. The respective sera samples were examined for various biochemical assays by using Microlab 300, Merck, Germany. Different biochemical parameters including total cholesterol, LDL, HDL, triglyceride, glucose & insulin levels and antioxidant status were accessed using respective commercial kits. Likewise, kidney function test were performed to evaluate the renal modulating aspects of drinks alongside liver functionality for safety reasons. The entire biological trial was repeated to draw an unambiguous assumption.

The details of these studies are herein;

### Study I: diabetic rats

In study I, high sucrose diet containing 40% sucrose was given to induce hyperglycemia in rats and determined its effect on serum glucose and insulin levels. Besides, effect of functional drinks on the induced syndrome was measured in each group at the termination of the study.

### Study II: obese rats

In study II, high cholesterol diet *i.e.* 1.5% of cholesterol along with high sucrose diet 40 % was given to the normal rats to induce hypercholesterolemia as well as

**Table 1.** Treatments for functional drinks.

| Treatments | Description |
|---|---|
| $T_0$ | Control |
| $T_1$ | Dietary Supplement (Catechins) @ 500mg/500mL |
| $T_2$ | Dietary Supplement (Theaflavin) @ 500mg/500Ml |
| $T_3$ | Dietary Supplement (Ginger freeze dried extract) @ 500mg/500Ml |
| $T_4$ | Dietary Supplement (Catechins+Theaflavin+Ginger freeze dried extract) @ 166+166+166mg/500Ml |

**Table 2.** Diet and treatment plan used for bio-efficacy trial.

| Diets | High Sucrose | | | | | High Cholesterol + High Sucrose Diet | | | | | $CCL_4$ Enriched Diet | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diabetic Rats | | | | | Obese Rats | | | | | Liver Injured Rats | | | | |
| **Groups** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **Drinks** | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ |

$T_0$: Control (Group rely on respective Experimental Diet+ without active ingredient) theaflavins supplementation @ 1g, $T_1$: Group rely on respective Experimental Diet+ Coriander seed supplementation @ 1g/Kg B.W , $T_2$: Group rely on respective Experimental Diet+ Black Cumin seed supplementation @ 1g/Kg B.W , $T_3$: Group rely on respective Experimental Diet+ Fenugreek seed supplementation @ 1g/Kg B.W , $T_4$: Group rely on respective Experimental Diet+ Coriander Seed+ Black cumin seed + Fenugreek seed supplementation @ 0.333+0.333+0.333g/Kg B.W

obesity. Periodic examination of rats was carried out to evaluate the induction of obesity. The functional drinks were provided to the rats concurrently to synchronize their effect on the respective group.

**Study III: liver malfunctioned rats**

In group III, rats were administrated on high $CCL_4$ diet @ 1% to induce liver malfunctioning with simultaneous intake of respective functional drink to test their effect on selected serum parameters.

Each group consist of 10 Sprague dawley rats in each. All the studies were independent having their own control and in all studies all the animal were provided respective treatments for a period of 56 days.

**Feed, water intake and food efficiency ratio**

The experimental rats were monitored for the feed and water intake throughout the trial. The gross feed intake of each group was calculated every day, excluding the spilled diet throughout the study period. The net water intake was also recorded on daily basis by measuring the difference in graduated bottles. The food efficiency rate was calculated by dividing the weight gain (g) by the total food intake (g) at the end of the experiments.

**Body weight gain**

The gain in body weight for each group of rats was monitored on weekly basis to estimate any suppressing effect of spices formulations.

**Serum lipid profile**

Cholesterol level of collected sera was measured by liquid cholesterol CHOD–PAP method according to the guidelines of Kim *et al.,* (5). Serum low density lipoproteins (LDL) were estimated following the protocol of Kim *et al.,* (5). Accordingly, the high-density lipoproteins (HDL) were assessed by Cholesterol Precipitant method (6). The triglycerides in the collected sera were measured by liquid triglycerides (GPO–PAP) method as described by Kim *et al.,* (5). Likewise, vLDL were calculated through dividing the triglyceride with 5.

**Glycemic indicators**

In each group, glucose concentration was estimated by GOD-PAP method as described by Katz [7], whereas, insulin level was estimated by following the instructions of Ahn (8).

**Indicators for hepatoprotective effect**

For liver soundness, alanine transferase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP) were estimated. The ALT and AST levels were measured by dinitrophenylhydrazene (DNPH) through Sigma Kits 58-50 and 59-50, respectively whereas; Alkaline Phosphatas-DGKC was used for ALP assessment. Moreover, α-Tumor necrosis factor (α-TNF), Fetoprotein, Total Body proteins, Albumin and Globulin were measured according the guidelines of (Gokul *et al.,* Mizejewski *et al., and* Bradford *et al.,*) (9, 10, 11).

**Indicators for oxidative stress**

The oxidative stress managing perspective were assessed by estimating the Total Antioxidant capacity (12), Glutathione assay (13), Xanthine oxidase (XO) and nitric oxide (NO) (14), TBARS (13), catalase and MDA (15)

**Statistical analysis**

The data regarding different treatments was obtained by applying completely randomized design (CRD) and further subjected to statistical analysis using Statistical Package (Microsoft Excel 2016 and Statistix 9.1). Level of significance was determined (ANOVA, LSD for comparison) using 2-factor factorial CRD where applicable following the principles outlined by Steel (16).

## Results

**Feed, Drink intakes & Food efficiency ratio**

The treatment imparted nonsignificant ($p \geq 0.452$) decline in feed intake of experimental rats feed on polyphenol based experimental diets in all studies. In this milieu, T1 imparted maximum decline followed by T4, T2 and T3. Similar non-momentous ($p \geq 0.125$) effect of treatment on drink intake was observed. The drink intake was higher in control group as compared to experimental treatment feed groups in respective studies. The weight of experimental rats has increased with the passage of time. The maximum enhancement in weight was observed in rats fed on high cholesterol diet in comparison with rats fed on other diets. However, the experimental diet rely group showed significantly ($p \geq 0.004$) less weight gain as compared to their respective controls.

**Indicators for Obesity**

***Body weight***

Final body weight at the termination of trial elucidated the weight management perspective of tested compounds. In this context, more pronounced effect

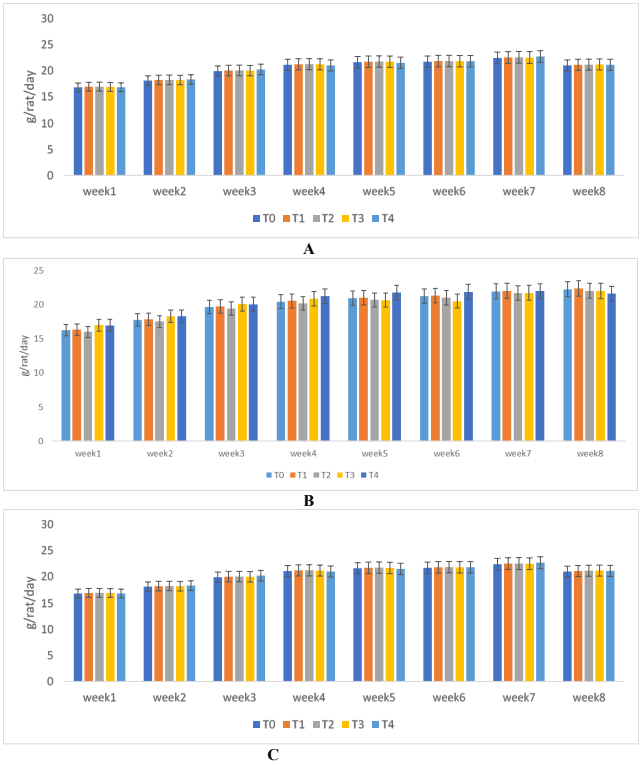


**Figure 1.** Feed intake of rats in all studies measured during first week to 8th week (g/rat/day). (A: fed with hyperglycemic diet; B: fed with high cholesterol diet+ Sucroose Diet; C: fed with High CCL4 diet).

was observed in obese rats where the experimental diets caused significant ($p \geq 0.002$) reduction in weight gain as compared to control. The most potent effect was observed for catechin based diet (T1) followed by theaflavin based (T2), The combination of both catechins and theaflavins (T4) and GFDE (T3) as compared to control. Similar significant ($p \geq 0.005$) effect was observed in other studies. However, the effect was less obvious as compared to obesity induce groups (Figure 3). In study I, Experimental diets T1, T2, T3 and T4 imparted 10.23, 12.01, 9.01& 11.80%, respectively as compared to control. Whereas, in obese rats T1 caused 16.18% reduction in weight gain higher then that of T2, T4 & T3 as 14.1, 15.01, 12.01% as compared to control, respectively (Figure 2). Likewise, the observed % decline in weight gain by T1, T2, T3 and T4 in Liver malfunctional rats were 9.36, 10.58, 7.65 and 9.12% as compared to control.

### *Blood lipid profile*

The treatments imparted significant ($p \geq 0.005$) reduction in cholesterol levels of experimental animals in all studies nonetheless, more pronounced effect was noticed in obese rats. In diabetic rats, the cholesterol level varied significantly between control to experimental treatments from 125±5.9 to 113.73±6.8 mg/dL in T0 and T4, respectively. In obesity induce experimental rats, the effect was more pronounced and maximum decline in cholesterol was observed in rats fed on catechin based diet (T1) as compared to the rest of the treatments. The observed cholesterol reductions by T1, T2, T3 & T4 were 13.36, 12.15,11.95 & 9.36 %, respectively as compared to control. However, in study III the recorded variation of cholesterol between control to experimental treatments was 101±3.6 to 93.97±3.7 mg/dL(Table 3).

The high cholesterol and sucrose diet brings abnormal changes in the lipid profile of the experimental rats characterized by substantial enhancement in LDL with decline in HDL which bring cascade of lipid related malfunctioning in the body. Similarly, the high atherogenic diet of current study caused significant suppression in the HDL levels of experimental rats however, the polyphenol based treatments caused momentous uplift in this trait. The minimum HDL level was noticed in control $T_0$ that significantly improved in $T_3$, $T_2$ and $T_4$ in study I. Likewise, in study II & Study III maximum increased was exhibited by $T_1$ (5.25 & 5.12 %) trailed by $T_4$ (4.56 & 2.36), $T_2$ (4.45 & 2.01%) and $T_3$ (2.01 & 1.96%) as compared to control.

LDL oxidation is the mandatory phenomenon as a response of high cholesterol diet consumption. In current study, LDL levels increased abnormally in rats rely on high lipid diet however, the polyphenols based

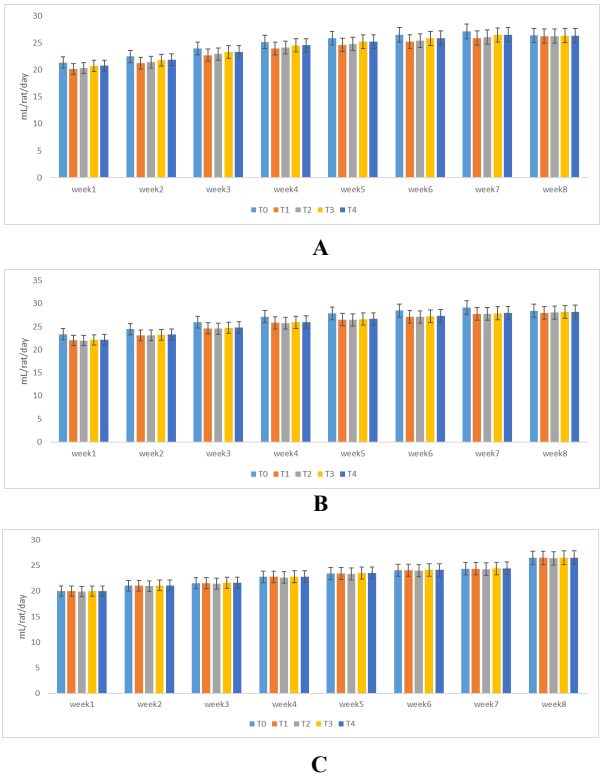


**Figure 2.** Drink intake of rats in all studies measured during first week to 8th week (mL/rat/day). (A: fed with hyperglycemic diet; B: fed with high cholesterol diet+ Sucroose Diet; C: fed with High CCL4 diet).

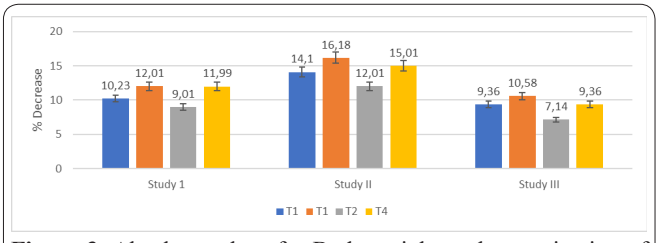


**Figure 3.** Absolute values for Body weight at the termination of the study (56th days). The experimental diets T0 (Group rely on Basic Experimental Diet), T1 (Group rely on Basic Experimental Diet+ Drink containing catechins @ 1g) T2 (Group rely on Basic Experimental Diet+ Drink containing theaflavins @ 1g) & T3 (Group rely on Basic).

intervention significantly ameliorate this abnormal elevation from 73.00±2.51 mg/dL ($T_0$) to 62.40±2.67, 64.07±2.69 65.72±2.49 & 63.40±2.47 mg/dL in Catechins ($T_1$), theaflavins ($T_2$), GFDE ($T_3$) and Theaflavin + Catechin + GFDE ($T_4$), respectively. Similar trend was apprehended in other studies, the highest LDL was observed in control which significantly reduced by the experimental treatments.

The means indicated that the triglycerides level in different groups of rats were significantly affected by treatments. In this context, maximum effect was exhibited by catechin based diet followed by theaflavin, the combination of theaflavin, catechin and GFDE and least effect was observed for GFDE. The recorded decline in TG levels in obese rats were Theaflavin ($T_1$) led to 8.56 & 8.54 % reduction however $T_4$ (Theaflavin + Catechin + GFDE), $T_2$ (Catechin) and $T_3$ (GFDE) caused 7.01 & 7.35 %, 6.36 & 6.30 and 4.58 & 5.01 % reduction, respectively. Similar diminishing trend in triglycerides with polyphenol-based treatments was observed in other studies however, the effect was less as compared to obesity induce rats.

Likewise, Control showed highest VLDL in both studies however, the polyphenol-based interventions caused momentous ($p \geq 0.014$) decline in this treat.

***Glycemic responses***

The glycemic responses including glucose and insulin were abnormally modulate in obesity and diabetic conditions. However, the treatments caused momentous ($p \geq 0.005$) modulations in glucose and insulin levels of rats in all studies. It is also worth mentioning that the more potent effect of treatments was observed in Study-I followed by Study-II and Study-III. In diabetic rats, substantial decreased by 10.25, 9.96, 6.63 and 11.23 % in $T_1$, $T_2$, $T_3$ and $T_4$ groups was observed, respectively as compared to control. Likewise, trend was observed for other studies, the maximum effect was caused by Theaflavin + Catechin +GFDE ($T_4$) followed by catechin ($T_1$), theaflavin ($T_2$) and GFDE ($T_3$) Table 3.

The high sucrose diet (Study-I) induce abnormalities in Insulin levels in response to abnormal glucose production. The experimental treatments significantly uplift this trait. In this context, $T_4$, rely group showed maximum increase followed by $T_1$, $T_2$ and $T_3$. Similar trend was recorded in all other studies by the treatments.

**Indicators for antioxidant indices**

The oxidative stress is among the major causative factors for the onset of cascade of abnormal metabolic responses that triggers the initiation of obesity, diabetes and other maladies. For the estimation of antioxidant potential of tested compounds, the total antioxidant activity through glutathione, TAC and TBARS assay were measured. It is worth mentioning that again combination of polyphenols (T4) performed better as compared to their alone treatments. In study I, the minimum glutathione level was noticed in $T_0$ (42.12±1.38 mg/L) that momentously elevated in $T_1$ (9.96%), $T_2$ (7.69%), $T_3$ (5.56%) and $T_4$ (10.02%). Likewise, in study II, the glutathione in $T_4$ (13.56%) was substantially increased as compared to $T_0$ (39.29±1.29 mg/L). Whereas, the recorded enhancement in T1, T2 and T3 were 12.25, 10.02 & 8.56 %, respectively. Similarly, in study III, glutathione values were uplifted from 41.23±1.22 mg/L ($T_0$) to 14.25% ($T_4$), 13.25 % ($T_1$), 11.63% ($T_1$) and 9.36 % ($T_2$), respectively (Table 3). Similar significant enhancement was observed in TAC in all studies by the treatments and order of effectiveness was Theaflavin + Catechin +GFDE >Catechins >Theaflavins>G.F.D.E. It is also well elaborated by results that maximum uplift was noticed in hyperlipidemic rats followed by hyperglycemic and liver malfunctional rats. Likewise, all the treatments caused significant diminished in elevated TBARS & MDA levels and maximum diminished was observed in study-II followed by Study-I and Study-III (Table 3).

**Indicators for hepatoprotective effect**

***Liver enzymes***

Oxidative stress induced structural and functional abnormalities in the liver that results the abnormal elevation in the enzymes like ALT, AST & ALP that used to evaluate the extent of damage. The Administration of the polyphenol based designer food caused significant ($p \geq 0.004$) diminished in this increase all studies nonetheless, the most potent effect was noticed in liver malfunctional and hypercholestrolemic rats. All the treatment exhibited the momentous effect however, the maximum effect was observed for T4 followed by T1, T2 and T3 in AST, ALP, GGT and bilirubin levels. It is also worth mentioning that the combination of all the polyphenols exhibited better effect in oxidative stress related pathogenies owing to diverse chemistry and mechanistic concerns. The order of effectiveness in all parameters was T4 >T1>T2>T3 (Table 3).

***α-Tumor necrosis factor (α-TNF), fetoprotein, total body proteins***

The utilization of CCL4 for liver damage caused abnormal increase both in α-TNF and Fetoprotein levels of rats moreover, abnormal levels of both were also observed in other studies where rats were administrated with high cholesterol and sucrose diet. The polyphenols-based interventions caused momentous reduction in these abnormal traits. The maximum values of α-TNF and Fetoprotein were exhibited in control group (T0) that significantly diminished in rats rely on Theaflavin + Catechin +GFDE ($T_4$) followed by catechin ($T_1$), theaflavin ($T_2$) and GFDE ($T_3$). Likewise, a significant diminished ($p \geq 0.004$) were observed in total body protein, Albumin and Globulin levels of rats in control group of all studies (T0) that uplifted at the termination of trial by the experimental treatments showing their oxidative stress related managing prospective (Figure 4).

**Discussion**

In the present study we might be first time evaluate the therapeutic effect of catechin, theaflavins and G.F.D.E based designer drink against obesity, diabetes and hepatic abnormalities. Our outcomes revealed that the catechin & theaflavin performed better against obesity and related complication owing to their modulating effect against fatty acid synthesis enzyme (FAS). However, the combination of all the polyphenols proved effectual against diabetes and hepatic injury may be owing to

**Table 3.** Effect of supplemented diet on serum triglycerides (TG), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), total cholesterol (TC), glucose, insulin, serum liver enzyme and antioxidant status in hyperglycemic, obese and liver malfunctional rats at the termination of the study.

| Parameters | Base line trend | Treatments | Study I | Study II | Study III |
|---|---|---|---|---|---|
| | | T0 | 125±5.9 | 155±4.9a | 101±3.6a |
| | | T1 | 112.88±4.7 | 134.29±5.9bc | 93.91±3.9bc |
| **Cholesterol mg/dL)** | 77.80±3.56 | T2 | 114.13±4.4 | 136.47±5.2d | 94.30±4.6d |
| | | T3 | 116.71±5.2 | 140.49±5.4b | 95.71±3.8b |
| | | T4 | 113.73±6.8 | 59±1.8cd | 30±0.9cd |
| | | T0 | 45±1.8 | 62.09±2.1b | 30.90±0.9b |
| | | T1 | 46.40±1.2 | 61.62±1.9a | 30.60±1.4a |
| **HDL (mg/dL)** | 32.56±3.56 | T2 | 46.33±1.9 | 60.18±2.3a | 30.30±1.8a |
| | | T3 | 45.50±1.7 | 61.69±1.9a | 30.70±1.9a |
| | | T4 | 46.35±1.8 | 61.71±1.62a | 40.02±0.99a |
| | | T0 | 63.00±1.80 | 73.00±2.51[a] | 28±1.86a |
| | | T1 | 55.91±1.26 | 62.40±2.67[b] | 25.88±1.34c |
| **LDL-C (mg/dL)** | 26.56±3.56 | T2 | 56.72±2.09 | 64.07±2.69[c] | 26.53±1.36c |
| | | T3 | 58.82±1.92 | 65.72±2.49[a] | 27.12±1.49b |
| | | T4 | 56.37±1.29 | 63.40±2.47[c] | 26.14±1.23c |
| | | T0 | 78±3.5 | 105±3.55a | 63±2.96a |
| | | T1 | 73.60±3.59 | 96.01±3.54b | 59.71±2.05b |
| **Triglyceride(mg/dL)** | 58.26±3.56 | T2 | 74.44±2.83 | 98.32±3.18b | 60.25±2.32b |
| | | T3 | 75.49±3.02 | 100.19±4.48b | 60.97±2.26ab |
| | | T4 | 74.09±2.61 | 97.63±3.52b | 59.84±1.85b |
| | | T0 (control) | 120±3.80 | 131±2.85a | 95±1.65a |
| | | T1 | 107.7±4.52 | 118.30±3.65bc | 88.70±1.94b |
| **Glucose (mg/dL)** | 79.63±3.72 | T2 | 108.04±3.65 | 119.78±2.45cd | 90.01±1.56bc |
| | | T3 | 112.04±3.65 | 123.12±2.80b | 91.84±1.52b |
| | | T4 | 106.52±4.65 | 117.88±2.36d | 88.25±1.58c |
| | | T0 (control) | 7.12±0.31 | 8.16±0.27a | 8.56±0.37b |
| | | T1 | 7.44±0.34 | 8.46±0.29a | 8.91±0.32a |
| **Insulin (µU/Ml)** | 6.23±0.56 | T2 | 7.40±0.38 | 8.42±0.25a | 8.86±0.39a |
| | | T3 | 7.33±0.37 | 8.40±0.31a | 8.84±0.34a |
| | | T4 | 7.47±0.32 | 8.50±0.35a | 8.93±0.31a |
| | | T0 (control) | 112.02±3.01 | 140.25±3.54a | 152.02±2.54a |
| | | T1 | 101.65±3.05 | 119.82±2.45ab | 125.75±2.87b |
| **AST(IU/L)** | 101.25±2.36 | T2 | 102.40±2.85 | 122.60±2.54 | 128.83±2.14b |
| | | T3 | 103.89±2.68 | 125.87±2.09ab | 130.70±2.69b |
| | | T4 | 99.41±2.54 | 117.45±2.65b | 124.27±3.65b |
| | | T0 (control) | 50.25±3.65 | 60.25±2.21a | 63.12±2.69a |
| | | T1 | 46.31±2.98 | 54.44±3.25b | 54.20±2.48b |
| **ALT(IU/L)** | 40.12±1.01 | T2 | 47.61±3.62 | 55.62±3.21b | 56.65±3.22b |
| | | T3 | 48.22±3.05 | 56.25±2.12b | 57.40±2.65b |
| | | T4 | 45.93±3.15 | 54.21±2.68b | 53.30±3.25b |
| | | T0 (control) | 154.02±0.25 | 225.32±1.98a | 240.15±2.32a |
| | | T1 | 144.22±1.25 | 202.22±3.65bc | 208.88±3.25bc |
| **ALP(IU/L)** | 130.24±2.01 | T2 | 147.47±2.05 | 204.90±2.98c | 216.08±2.34cd |
| | | T3 | 149.09±2.62 | 206.73±2.68b | 217.67±2.42b |
| | | T4 | 142.85±2.74 | 198.16±3.58d | 205.28±2.65d |
| | | T0 (control) | 42.12±1.38c | 36.32±1.33d | 37.14±1.18d |
| | | T1 | 46.31±2.11b | 40.76±1.91bc | 42.06±2.11bc |
| **Glutathione(mg/L)** | 55.12±1.01 | T2 | 45.35±1.47a | 39.95±1.60ab | 41.45±1.54ab |
| | | T3 | 44.46±2.34b | 39.42±2.1c | 40.61±1.34c |
| | | T4 | 46.34±1.41a | 41.24±2.7a | 42.43±2.11a |
| | | T0 (control) | 0.30±0.001c | 0.34±0.01d | 0.32±0.01d |
| **Total antioxidant activity (mmol Trolox equivalent/L)** | 0.3±0.001 | T1 | 0.27±0.02b | 0.49±0.21bc | 0.46±0.02bc |
| | | T2 | 0.29±0.016a | 0.52±0.01ab | 0.61±0.05ab |
| | | T3 | 0.23±0.03b | 0.42±0.02c | 0.41±0.04c |
| | | T4 | 0.25±0.005a | 0.60±0.01a | 0.59±0.02a |
| | | T0 (control) | 1.05±0.25a | 1.19±0.65a | 1.29±0.24a |
| | 1.04±0.005 | T1 | 1.0±0.95b | 1.07±0.68b | 1.15±1.25b |
| **Bilirubin** | | T2 | 1.018±0.35a | 1.08±0.32b | 1.17±1.54b |
| | | T3 | 1.02±0.35b | 1.11±1.02a | 1.19±1.54b |
| | | T4 | 0.98±0.54c | 1.06±0.65c | 1.13±0.69c |

| | | | | | |
|---|---|---|---|---|---|
| **Urea** | 22.60±0.004 | T0 (control) | 22.63±0.82 | 28±1.21a | 30±1.22a |
| | | T1 | 22.17±0.83 | 25.71±1.32b | 27.89±1.43ab |
| | | T2 | 21.84±0.74 | 25.47±1.43b | 27.47±1.37ab |
| | | T3 | 22.29±0.83 | 26.23±1.45b | 28.46±1.39ab |
| | | T4 | 21.94±0.69 | 24.65±1.36c | 26.99±1.52b |
| **Creatinine** | 0.80±0.001 | T0 (control) | 0.80±0.025a | 0.94±0.032a | 1.01±0.037 |
| | | T1 | 0.78±0.031b | 0.92±0.028ab | 0.95±0.031a |
| | | T2 | 0.77±0.027b | 0.91±0.029ab | 0.93±0.041b |
| | | T3 | 0.78±0.032b | 0.93±0.42a | 0.97±0.036a |
| | | T4 | 0.77±0.023b | 0.90±0.052b | 0.92±0.039b |
| **TBARS** | 8.89±0.003 | T0 (control) | 8.25±2.42a | 10.25±3.65a | 9.65±2.98a |
| | | T1 | 7.48±2.21ab | 9.19±3.87c | 8.48±2.65b |
| | | T2 | 7.54±2.65ab | 9.40±3.95b | 8.69±2.45b |
| | | T3 | 7.62±2.87ab | 9.53±4.01b | 8.96±2.47b |
| | | T4 | 7.25±2.98b | 8.78±2.89d | 8.17±2.89c |

Values are mean ± SEM (n = 10) : One way anova was applied to check the overall behavior of the study parameter to elaborate the effect of treatments on selected parameter of rats at the termination of study. To evaluate the differences among the mean LSD test was applied. Values in same column within each parameter with different letters were significantly different from each other ($p \leq 0.05$). Study I: Hyperglycemic rats, Study II : obese rats,Study III: Liver malfunctional rats. $T_0$: Control,$T_1$: Drink containing catechins, $T_2$: Drink containing theaflavins, $T_3$: Drink containing ginger freeze-dried extract (gingerol), $T_4$: Drink containing catechins+theaflavins+ginger freeze dried extract (gingerol).

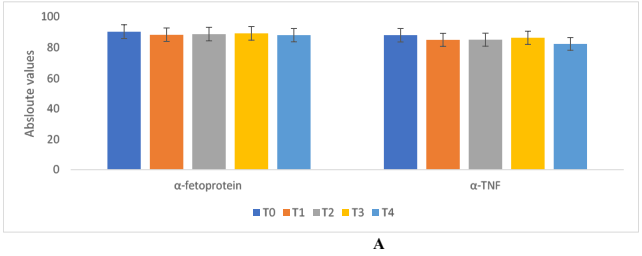


**Figure 4.** Absolute values (all studies) for indicators for liver health and body proteins of at the termination of study (56th days). The experimental diets T0 (Group rely on Basic Experimental Diet), T1 (Group rely on Basic Experimental Diet+ Drink containing catechins @ 1g) T2 (Group rely on Basic Experimental Diet+ Drink containing theaflavins @ 1g) & T3 (Group rely on Basic Experimental Diet+ containing ginger freeze dried extract (gingerol) @ 1g) and T4 (Group rely on Basic Experimental Diet+Drink containing catechins+theaflavins+ginger freeze dried extract (gingerol @33+33+33g, respectively) were given through entire study period. Values are mean ± SEM (n = 10) and level of significance were determined at ($p \leq 0.05$).

their greater antioxidant potential and structural variations. The polyphenol rich products have been reported to hamper the feed and drink intake owing to their effect on appetite and satiety. The less feed consumption in rats relies on tea polyphenols is documented by the findings of numerous earlier studies that emphasis that the satiety and improved feed efficiency are the possible reasons behind this trend (17).

Globally, the tendency to consume monotonous and energy dense diet coupled with lack of exercise initiate the production of oxidative stress that triggers the cascade of different maladies leading by obesity, diabetes and etc. Furthermore, oxidative stress caused structural and functional abnormalities in vital body systems like liver and kidney (18). Different bio-evaluation studies narrated the positive role of tea polyphenols like theaflavins and catechins to control obesity and hypercholesterolemia. Mechanistically, it stimulates the lipid metabolism and hinders the activities of gastric and pancreatic lipases. Additionally, it increases thermogenesis and suppresses the fatty acid synthase enzyme (FAS). In case of hypercholesterolemia, black tea polyphenols reduce plasma cholesterol, triglycerides and LDL by modulating the liver LDL receptors and cholesterol synthesis inhibitors (19, 20).

The formation of emulsion droplets enhances the surface for lipase absorption, that has an important role in lipid digestion that contribute towards the obesity. In this context, tea polyphenols (theaflavins, catechins and thearubigins) inhibits the formation of emulsion droplets and thus reduces the surface for lipase absorption thus helpful in obesity management. Theaflavins with galloyl moieties inhibited pancreatic lipase activity in vitro with an IC50 of about 0.5 mg/ml. This inhibition of lipase leads to lesser absorption of hydrolytic products of fat into plasma (21). A study showed the mice that consumed a standard diet or high fat diet containing 5% BTPE showed a decrease in body weight gain by 32% due to the inhibition of intestinal lipid absorption. Likewise, FAS was reported to play an important role in regulating feeding habits. Polyphenols like theaflavins and catechins are FAS inhibitors and they reduce food intake and body weight and triglyceride blood levels (2). Another important mechanism by which polyphenols are major maneuver to regulate the obesity is their ability to inhibit the activation of nuclear transcription factor Kb (NF-kB), thus preventing NF-kB from inhibiting PPARα to regulate lipid metabolizing enzymes and increasing fatty acid oxidation. PPARγ is present in muscles and adipocytes and it stimulates lipid uptake and lipogenesis by adipose tissues. Tea polyphenols also inhibits the adipogenic transcription factor PPARγ

that leads to weight reduction (22). Monocyte chemoattractant-1 (MCP-1) has a role in the development of obesity (21). Plasma MCP-1 levels are up-regulated in obese patients and obese mice that were fed high fat/ high sucrose diet. Black tea polyphenols reduced MCP-1 concentrations in epididymal fat by inhibiting the expression of MCP-1 gene (23).

Likewise, catechins and theaflavins suppresses the intestinal cholesterol absorption and micelle formation by inhibiting the incorporation of cholesterol in mixed micelles (21) Black tea polyphenol extract (BTPE) prevents diet- induced obesity by suppressing the intestinal lipid absorption. The main active component of BTPE is polymerized polyphenol fraction (24).

Polyphenols ameliorates high triglycerides by suppressing lipid accumulation, reduced fatty acid synthesis and enhanced fatty acid oxidation through activation of LKB1-AMPK pathway. The anti-obesity effect of tea polyphenols are further verified by the findings of Jina *et al* (25), examined the obesity reducing potential of black tea purified theaflavins in obese rats. Likewise, the outcomes of many scientific exploration depicted the beneficial impact of ginger polyphenol with special reference to gingerol against many physiological disorders. In this context, Al-Amin *et al.,* (26) carried a 7-week study by involving streptozotocin (STZ)-induced diabetic rats to evaluate the therapeutic potential of ginger polyphenol in obesity and related comorbidities. The rats were administrated raw ginger extract @ of 100 ppm and they observed marked decline in all obesity related parameters with marked decline in serum glucose, cholesterol and weight gain. Likewise, Kadnur and Goyalet (27) also described marked reduction in cholesterol, body weight, insulin resistance and glucose level by consuming ginger freeze dried powders. A similar lipid and weight diminishing pattern was observed by different researchers groups like Misawa *et al.,* (28) & Akash *et al.,* (29) observed modulation in diabetes and obesity markers in animals by the administration of ginger extracts.

Diabetes prevalence was increased at an alarming rate due to the unhealthy dietary pattern combined with metabolic dysfunctions and obesity caused immense health care burden on developing economies and Pakistan is no exception. Numerous previous studies have explored the beneficial role of polyphenols for the management of diet and life style induce diabetes. Polyphenols have credentials as strong glycemic management agent owing to the higher antioxidant potential and ability to modulate various glycemic management biomarkers. Catechins, theaflavins and ginger (gingerol) have widely explored for their diabetes management capacity as alone. However, as leading polyphenols their combination utilization as intervention has not been yet adapted.

Substantial evidences have divulged the role of tea as an anti-diabetic agent due to its strong antioxidant potential. The tea allied antioxidants attenuate hyperglycemic state by modifying the glucose metabolism, affirmative influence on insulin secretion and absorption through the modulation of ß-cells (30). Being a strong antioxidant agent, tea polyphenols have ability to ameliorate the chronic oxidative stress on the pancreatic beta-cells when they have low levels of antioxidants in plasma is indicated as a risk factor for diabetes and a contributing factor for the development of diabetic complications like atherosclerosis and microvascular complications (31). Glucose transporters (GLUTs) are important in controlling blood glucose concentrations. Black tea polyphenols improve translocation of GLUTs in skeletal muscles where GLUTs uptake glucose and reduce postprandial hyperglycemia (32). In the skeletal muscles, glucose uptake and glycogen synthesis are promoted by insulin. But glucose uptake and glycogen synthesis abnormalities occurs in diabetes leading to hyperglycemia and other complications (33). Tea polyphenols translocate GLUTs in L6 myotubes through phosphatidylinositol 3-kinase (P13k) and AMPK dependent pathways. Another possible route is the impact of black tea polyphenols on activity of amylase enzyme. they resulted in slow breakdown of starch thereby control the sudden rise in glucose. In a randomized cross over trial, 16 subjects were provided 75 g of glucose and water daily with simultaneous provision of 3 g instant black tea and attributed this effect to tea polyphenols ability to stimulate the pancreatic enzymes that enhance β-cell ability towards insulin (34). Likewise, ginger is also exhibited strong antidiabetic activity that is further illuminated through the earlier findings of Shidfar *et al.,* (35) probed the beneficial effect of ginger administration on the glycemic biomarkers in Iranian type 2 diabetes patients. The outcomes suggested that the 90 days ginger provision caused marked improvements in total antioxidant capacity, glucose level and insulin signaling. The trend was further verified by the outcomes of Daily *et al.,* (36) conducted the meta-analysis of five randomized clinical trials (RCTs) and probed the effect of ginger on fasting blood glucose and insulin, homeostatic model assessment (HOMA)-insulin resistance (IR), and hemoglobin A1c (HbA1c) levels.

Liver is an important organ for endogenous and exogenous substances to be detoxified and deposed. However, amplified production of reactive oxygen species owing to poor dietary and life style perspective accelerate the liver abnormalities. The liver functioning enzymes like AST and ALT & GGT levels predict the soundness of the liver. In liver malfunctioning these leaked into the serum and showed early sign of liver injury that increase during hypercholesterolemic and hyperglycemic phase (37-39). Among the different copping strategies, antioxidant based dietary interventions have gained paramount attention among the scientific community and consumer as intervention against oxidative stress related maladies (40). Plant based bioactive compounds have been documented to provide ameliorated role against abnormal liver functioning enzymes and tea and ginger are examples of such food that naturally loaded with such bioactive moieties. Ginger has been extensively studied for its therapeutic potential in hepatotoxicity and outcomes delineated reduced level of liverfunctioning enzymes after treated with ginger based interventions (37-39). The liver enzymes restoration effect of ginger in current study are in corroborated with the earlier findings of Choudhary and Devi (41), they carried a model feed trial involving the experimental rats. The outcomes showed that the ginger based treated groups showed a significant improvement in the abnormal levels of AST, ALT, ALP and π-GT elevated after

aspartame treatment. Likewise, α-fetoprotein, and total necrosis factor (TNF) levels were also significantly reduced through ginger treatment. They ascribed that the therapeutic worth of ginger is a function of their strong antioxidant potential that induced balance in internal antioxidant status through accelerating the endogenous antioxidant production. Likewise, Atta *et al* (42) also investigated the effect of ginger and chicory on liver malfunctioning induced by $CCL_4$ Injection and observed the restoration effect of ginger. Later one of the scientists group, Dong *et al.,* (43) elaborated the role of ginger in CCl4 induced liver malfunctioning. Alongside the observing the liver enzymes they were well interested to evaluate the role of TNF in the progress and rectification of liver pathogenesis. They expressed that besides lowering the concentration of ALT and AST the level of TNF was also reduced. Moreover, they were also noticed less abnormities where TNF level was present in normal concentration. Likewise, regular tea consumption may protect liver by modulating the antioxidant enzymes and lipid peroxidation via enhanced glutathione peroxidase (GSH-Px), superoxide dismutase and catalase. Under oxygen deficient conditions, hydrogen peroxide is produced that causes toxicity and initiates the cascade of undesirable events. The antioxidant enzyme glutathione converts hydrogen peroxide into water and reduces the thiobarbituric reactive substances (TBARS) production thus acts as safeguard against lipid peroxidation (19). Similarly, Łuczaj and Skrzydlewska(44) narrated from research that performance of glutathione and other antioxidant enzymes is reduced in alcohol intoxicated rats. Results revealed that glutathione activity raised from 16.27 ± 0.68 to 18.12 ± 0.74 nmol/mL indicated 11.01% increase in normal rats. While in alcohol intoxicated rats, glutathione level increased from 13.25 ± 0.89 to16.84 ± 0.79 nmol/mL exhibited 22.3% enhancement. One of the researchers groups, Siddiqui *et al* (45) evaluated black tea extract for the enhancement of serum glutathione in oxidative damaged rodents. The glutathione contents increased 26.02% by administrating black tea @ 5%.

The outcomes of the bio-evaluation study on Sprague Dawley rats delineated that the polyphenol based dietary interventions not only alone but in combinations exerted beneficial impact to mitigate the diet induced obesity, diabetes and liver insult owing to its strong antioxidant potential.

## Competing interests

The authors declare that they have no competing interests.

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