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ORIGINAL ARTICLE **OPEN ACCESS**

In Vitro, In Vivo Antioxidant and Antidiabetic Activity of Catechin Extract Extracted from Green Tea (*Camellia sinensis*)

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ABSTRACT

Millions of people around the world are affected by diabetes mellitus (DM). Medicinal plants have also become more interested in use of natural medicines and supplements with less adverse effects with lower cost. In this study catechin extract of camellia sinensis was taken to confirm inhibition of in vitro digestive carbohydrate enzymes and in vivo type 2 diabetes in rat model. Extraction isolation and phytochemical investigation was performed and extract was taken for in vitro antioxidant DPPH assay then in vitro antidiabetic assay following in vivo animal study. The findings demonstrate that catechin extract isolated from camellia sinensis shows strong inhibitory activity against α-amylase with IC 50 values 66.12 and 40.23 for the α-glucosidase enzyme. Antihyperglycemic property of catechin extract camellia sinensis was found to be satisfactory. There have been noted reductions in total serum cholesterol lipid peroxidation, and an increase in serum glutathione concentration. In addition, serum creatinine, AST, and ALT levels has been checked. Total phenolic also showed a similar trend with a significant reduction in ALP and phosphatase were significantly decreased when compared to untreated DM. TPC and TFC and potent antioxidant activity of (122.57 ± 8.88mg gae/g), and (122.99 ± 21.24mg qe/g extract) are believed to have helped in the anti-diabetic effect of the extract. This study shows that catechin extract isolated from camellia sinensis is a natural source of antioxidants that can be used in the cause, cure, and management of diabetes.

Keywords: *Catechin Extract, Antidiabetic Activity, In Vitro Assay, In Vivo Activity*

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INTRODUCTION

Diabetes mellitus leads to an incapacity of the body to make or react with insulin and therefore maintain appropriate amounts of sugar in the blood. This results in several adverse effects and complications such as neuropathy, nephropathy, retinopathy, hyperthyroidism, hypertension, and arteriosclerosis. It is a chronic, lethal, and noncommunicable condition known as diabetes mellitus (DM) [1]. It is a consequence of elevated levels of blood sugar, also known as, hyperglycemia. Diabetes mellitus responsible for hyperglycemia a major contributor to the formation of oxidative stress[2-5]. It actively participates in the impediment of insulin action, insulin function and its secretion. There is considerable evidence that oxidative stress majorly occurs due to relationship between stress and DM complications. In addition, antioxidants play a very good role in treating complications of diabetic patients. Oxidative stress arises in the body due to generation of free radicles which leads to cellular damage and by this destruction of pancreatic beta cells. Chemically, the medicinal plant comprises numerous chemical compounds majorly flavonoid, phenols, gallocatechins [6].

This study has undertaken antioxidant effect of catechin extract isolated from camellia sinensis commonly known as green tea. Green tea contains active ingredients called catechins that can be used for treatment of diseases. Life itself cannot exist without glucose. In fact, the level of glucose in serum is considered hyperglycaemia when above the threshold. Polyphenol rich green tea could be characterized as an important dietary source of polyphenols, especially flavonoids. Catechins are typical flavan-3-ols, which are the major flavonoids in green tea. The major catechins are the epigallocatechin 3-gallate, Epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin 3 gallate (ECG), epicatechin (EC) in descending dosage order. Green tea extract and its catechins do not show any effects on GP. Here, we demonstrate in vitro suppression of alpha- glucosides, alpha amylase carbohydrate digestive enzyme by green tea extract and in vivo antidiabetic activity [7-12].

MATERIAL AND METHODS

Folin–Ciocalteu reagent, gallic acid butylhydroxytoluene,1,1-Diphenyl-2 picrylhydrazyl (DPPH) ,acarbose , p-nitrophenyl-α-D-glucopyranoside (pNPG), quercetin, potassium chloride, 3,5-dinitrosalicylic acid (DNS), starch, α-amylase from porcine pancreas, α-amylase and α-glucosidase. Streptozotocin was purchased from SRL MUMBAI. Metformin was given as a gift sample from Wockhardt Ltd, Aurangabad, Maharashtra.

Extraction of Catechin from *Camellia Sinensis*

Catechin was isolated from green tea leaves by fractionating with chloroform and ethylacetate. 250 gm of green tea leaves were taken and kept for extraction with chloroform to remove chlorophyll and caffien. This de chlorophylled and decaffeinated extract was again extracted with ethylacetate. The ethylacetate fraction was kept for drying and dried powder was used for further phytochemical screening.

Phytochemical Screening [13-14]

Green tea catechin extract was under taken for phytochemical screening by performing test for various phytochemicals like alkaloid, flavonoid, phenols, saponins etc.

Test for Tannins: About 200 mg of the plant extract was boiled with 10 mL of distilled water; and 0.1% Ferric chloride was added to the mixture; which was then observed for blue-black coloration indicating the presence of tannins.

Test for Alkaloid: The extract of plant was dissolved in 100 mL of water, filtered, and cooked in steam with 2 mL of the filtrate and three drops of 1% HCl. 1 mL of the boiled mixture was mixed with 6 mL of the Mayer-Wagner reagent. The presence of a cream or brown-red colored precipitate specified the presence of alkaloids.

Test for Saponins: About 0.5 milliliters of the plant extract and 5 mL of dl water were combined and shaked. Afterwords, the formation of foam confirmed the presence of saponins.

Test for Flavonoids and Glycosides:200 mg of the plant extract was combined with 10 mL of ethanol and filtrated. 2mL of the filtrate, concentrated HCl, and magnesium ribbon were combined. The formation of a pink or red color shows the presence of flavonoids. Adding 1 mL of dl water and NaOH to 0.5 mL of crude plant extract, the formation of a yellowish color shows the presence of glycosides.

Test for Steroids: About 1 mL of the crude plant extract was mixed with 10 mL of chloroform and 10 mL of sulfuric acid, and the creation of the bilayer (red top layer and greenish bottom layer) reveals the existence of steroids.

Test for Phenols: About 1 mL of the extract was mixed with three drops of FeCl3.

Test for Triterpenoid: Salkowski test: Extract 5ml was mixed with CHCL3 2ml and conc sulphuric acid 3ml was prudently added to form a layer. A reddish brown coloration of the filter face was formed to show positive results for the occurrence of terpenoids of K2Fe (CN6). The formation of greenish-blue forms confirms the occurrence of phenol.

Total Polyphenol Content Determination: The polyphenol content was performed according to the Folin - Ciocalteu method described by Pham, Hong Ngoc Thuy et al. (2017) with some corrections. The extract was diluted to the appropriate concentration, then 0.5 mL of the diluted extract was added to a test tube and 2.5 mL of Folin-Ciocalteu reagent was added. The mixture was homogenized using a Vortex before adding 2 mL of 7.5% Na2CO3. Then, the reaction mixture was kept at room temperature for 30 min before measuring the absorbance (Abs) at 765 nm on a spectrophotometer. Results are reported as milligrams of Gallic acid equivalent (mg GAE)/g dry matter.

Total Flavonoid Content Determination: Flavonoid content was performed by chromogenic method with AlCl3 and Quercetin used as standard, in alkaline medium. Add 0.5 mL of the previously diluted solution to the test tube. Then, 0.1 mL of 10% AlCl3 solution and 0.1 mL of 1 M CH3COOK solution with 4.3 mL of distilled water, the mixture was shaken well and then stabilized at room temperature for 30 min. After 30 min, the absorbance was resolute by calculating the spectrometer, at 415 nm. Quercetin was used as the standard. The total flavonoid content (TFC) is presented as mg Quercetin weight per g extract (mgQE/g extract).

DPPH Radical Scavenging Activity: DPPH radical scavenging activity Antioxidant capacity was performed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method Bui, L. T. K. et al. (2019). Dilute the extracts to the appropriate concentration range with methanol and ascorbic acid solution (Vitamin C) as standards for comparison. The control sample changed the extract with methanol. Place 1 mL of extract (or ascorbic acid) of each concentration in a test tube respectively, then add 1 mL of DPPH solution (0.3 mM). After 30 min of incubation in the dark at given room temperature, absorbance was noted at 517 nm on a UV–Vis spectrometer. The antioxidant activity was calculated according to the formula:

% DPPH radical scavenging = (sample–negative control)/negative control) × 100.

Assessment of alpha amylase inhibition:The assay components are 120µL of 0.06 M potassium phosphate buffer, 20µL of enzymes, 40 µL of gymnemic acids within the concentration range of 20 – 100µg/ml. Then, after incubation, 40µL of substrate was added and distilled water was added to make the volume of samples

equal. Incubation took place in order to mix the reaction blend, which it lasted for 10-15 minutes at a temperature of 37°C. 40 microliters of DNS I reagent were added and the mixture was heated in a boiling water bath. A 540nm of measure was applied to the sample taken for absorbance by using ELISA reader. These consisted of plain uncompounded samples for use as controls. Aimed as the percent inhibition using the equation below,

$$\text{Inhibition (\%)} = \frac{\text{Abs (405) control} - \text{Abs (405) Test}}{\text{Abs (540) Control}} \times 100$$

Assessment of alpha glucosidase inhibition: Alpha glucosidase inhibition was estimated based on Ranilla et al. procedure with certain enhancements. It contained 20µl alpha-glucosidase 0.5 unit/ml; 120µl of 0.1M phosphate buffer (pH6.9) and 10µl test sample diluted as indicated. After adding a mixed solution into 96-wells place and further incubating it at 37° for 15min; the enzymatic reaction proceeded by addition of 20µL p-nitrophenyl-α-D-glucopyran. Then, the reaction was terminated using addition of 80ul of 0.2 M sodium carbonate solution and later absorbance reading was done in a microplate reader (BioTek XS2) at 405nm. Plant phytochemical was used as the lack of reaction system while α-glucosidase used without for calculation of the background absorbance. That is why the following formula was utilized to calculate the inhibitory rate for a herbal sample of α-glucosidase.

$$\text{Inhibition (\%)} = \frac{\text{Abs (405) control} - \text{Abs (405) Test}}{\text{Abs (540) Control}} \times 100$$

Experimental protocol: The test samples were catechin extract suspended in distilled water. The treatment regimen involved metformin in a dosage of 100 mg/kg and acted as the standard control. The test was done by oral route on all the sample. The administration of diabetes in rats was done by making use of 65 mg/kg of streptozotocin freshly dissolved into normal saline solution. The rats were fed with normal pellet diet immediately after ip injection of streptozotocin, they had unrestricted access to both food and water. Polydipsia and polyuria of mild degree were recognised as diabetes in rat. It was also established that after 3 days or 72 hrs of injection; the fasting blood glucose levels will be computed using the well accepted GOD/POD method with a commercial glucometer based on UV-visible spectrophotometer at 50 Diabetes rats were identified based on their fasting blood glucose which was greater than 180mg/dl.

Experimental Design:

Group no	Group Name	Receiver
I	Normal Control	Received only vehicle that is distilled water.
II	Diabetic control	rats received only vehicle that is distilled water
III	Herbal Drug 1	received Catechin extract (100 mg/kg/day p.o) suspended in distilled water
IV	Herbal Drug 2	received catechin extract (200 mg/kg/day p.o) suspended in distilled water
V	Standard	received Metformin (100 mg/kg p.o) suspended in 2% v/v Tween 80 solution

The grouping is arranged in such a way that group I and group II described as normal and diabetic control, group III and group IV as herbal extract receiver in doses 100mg/kg and 200mg/kg respectively, V group received standard drug i.e metformin 100mg/kg .Test sample was given accordingly and blood glucose level was checked on every alternate day like 1,3,5,7,9,till 21st day of antidiabetic study blood glucose measurement was done by using glucose testing kit.

Statistical Analysis

The statistical analysis data were articulated as mean ± standard error mean (sem). The significance of differences between the group was evaluated by applying one way and multiple way analysis of variance (ANOVA). The test followed by dunnet's test p values less than 0.05 were considered as significance. All data are articulated as the standard error of the mean. Comparisons between the control and treatment groups were made using analysis of variance followed by a student- newman-keuls t-test using the graph pad in stat statistical program. Analysis of all samples was calculated by, an associated probability (p value) of less than 5% ($p<0.05$) and it was considered significant.

RESULTS

Phytochemical Identification of catechin extract

Table 1. Phytochemical screening of four herbs aqueous extracts

Chemical components	*Camellia Sinensis* catechin extract
Tannins	+
Phenols	+
Anthraquinone	+
Flavonoids	+
Triterpenoids	-
Saponin	-
Alkaloid	-
(+) indicates present, (−) indicates absence.	

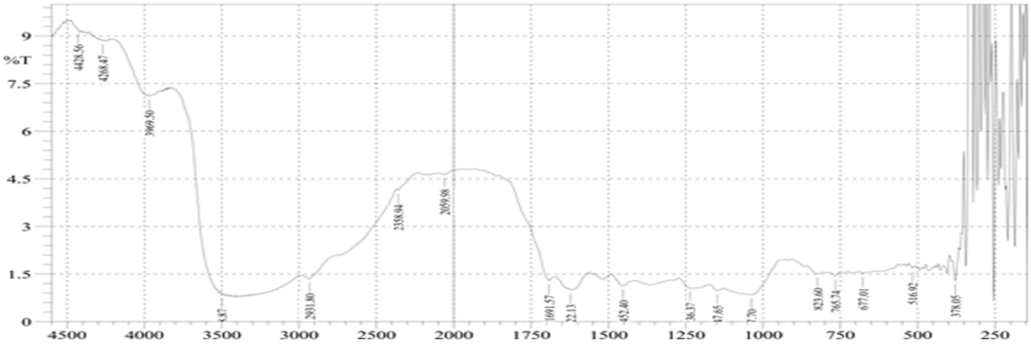

Figure I: IR spectra of catechin extract

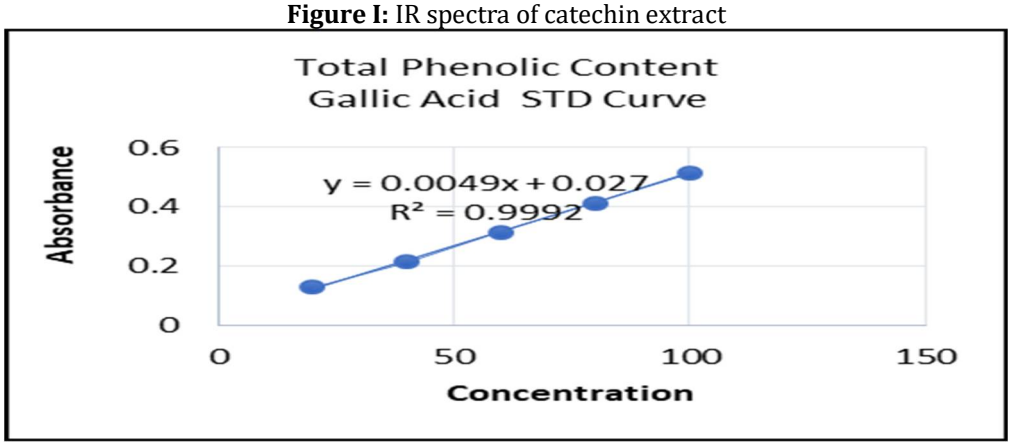

Figure II: Graphical Representation of TPC **(**Total Phenolic Content)

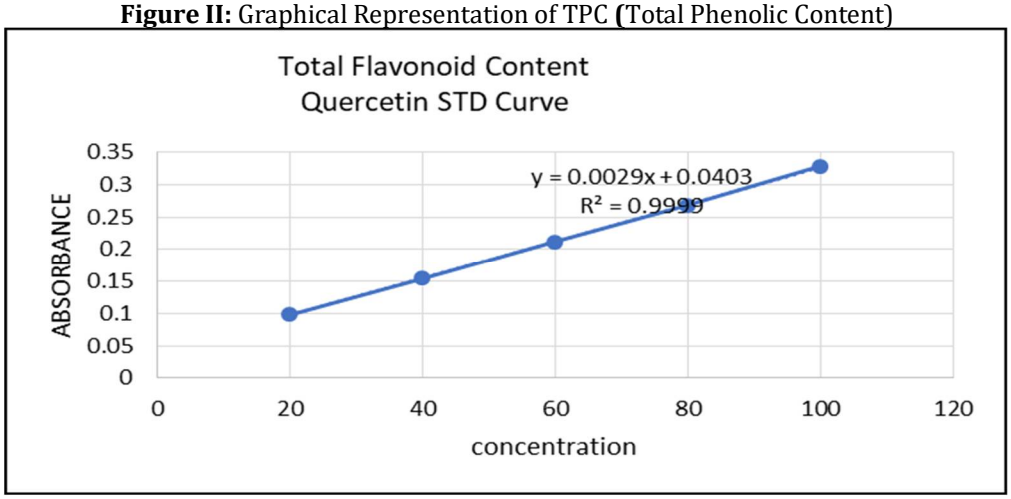

Figure III: Graphical Representation of TFC (Total flavonoid Content)

Table II. Total phenolic and flavonoid content of four herbs

Sample	Polyphenol (mg GAE/g)	Flavonoid (mg QE/g)
Camellia Sinensis catechin Extract	122.57 ± 8.88	122.99 ± 21.24

Table III and graph IV represent the alpha amylase inhibitory activity of catechin extract and Acarbose. Concentration dependent inhibitory effect of alpha amylase was maximum in 100µg/ml in both the

catechin extract and Acarbose with IC50 value of 66.12µg and 40.23µg respectively. Catechin extract shows low inhibitory effect when it is compared with Acarbose drug.

Table III: Effect of Catechin extract and Acarbose on α-amylase activity

Concentration (µg/ml)	Catechin Extract		Acarbose	
	% inhibition	(IC_{50})	% inhibition	(IC_{50})
20	20.60 ± 1.68	66.12	22.63 ± 0.78	40.23
40	28.47 ± 1.29		46.67 ± 1.76	
60	54.13 ± 1.63		52.30 ± 1.25	
80	51.63 ± 1.55		65.17 ± 1.89	
100	62.40 ± 1.64		79.87 ± 1.33	

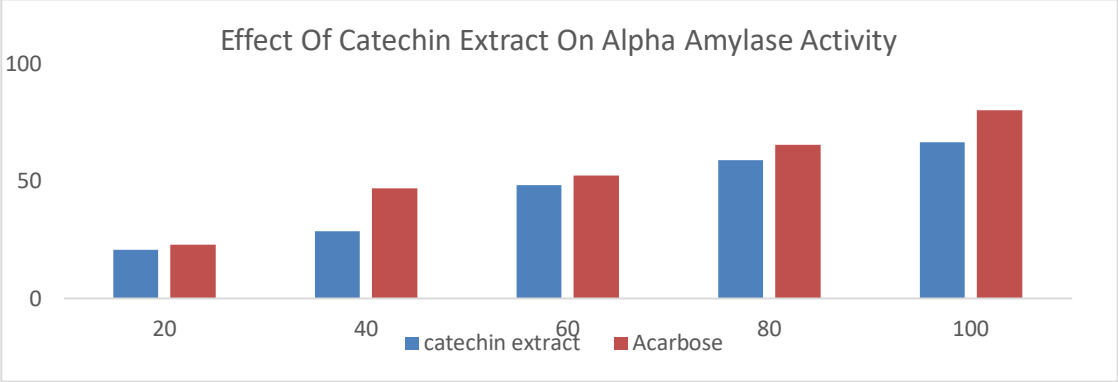

Figure IV: Effect of Catechin Extract and Acarbose on Alpha Amylase Activity

Table IV and Figure V shows the α-glucosidase inhibitory activity of Catechin extract and Acarbose. Concentration dependent inhibitory effect of α-glucosidase was shown maximum in 100µg/ml in both catechin extract and Acarbose with IC50 value of 66.77µg and 35.67µg. Catechin extract shows less inhibitory effect when compared to that of Acarbose drug.

Table IV: Effect of catechin extract and Acarbose on α- Glucosidase activity

Concentration (µg/ml)	Catechin extract		Acarbose	
	% inhibition	(IC_{50})	% inhibition	(IC_{50})
20	21.67 ± 1.44	66.77	23.63 ± 1.64	35.67
40	27.80 ± 1.59		55.97 ± 1.55	
60	44.10 ± 1.01		67.60 ±1.28	
80	51.43 ± 1.50		76.43 ± 1.40	
100	71.50 ± 1.32		90.20 ± 1.74	

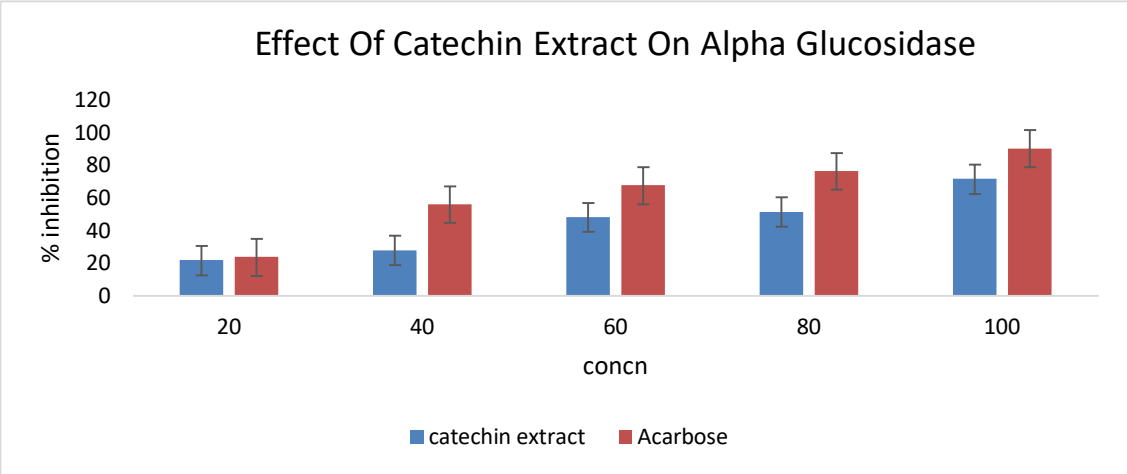

Figure V: Effect of Catechin Extract on Alpha Glucosidase

Animal Study Result

Upon administration of Catechin extract , significant changes were recorded in blood glucose levels, triglycerides, total cholesterol levels, urea and creatinine levels both in acute and in chronic study groups. It was observed that the higher dosage of Catechin extract exhibited increased reduction in the values of parameters compared to low dosage administration. The values of the glucose levels in blood observed by

treating diabetes induced rats with Catechin extract was comparable to the values obtained by treating with metformin. Recorded values showed a dose dependent reduction of blood glucose levels, total cholesterol, triglycerides and urea levels in the streptozotocin induced diabetic rats treated with Catechin extract.

Table V: Effect of catechin extract On Weight After Every 7 Days

DAYS	Normal	DC	Metformin 100 mg/kg	100 mg/kg Catechin Extract	200mg/kg Catechin Extract
0th day	164	166	170.4	163	161
7th day	168.2	169.2	172.4	165.8	178.2
14thday	213	226.2	220.8	221.4	220
21th day	228.4	243.6	225.8	221.4	225

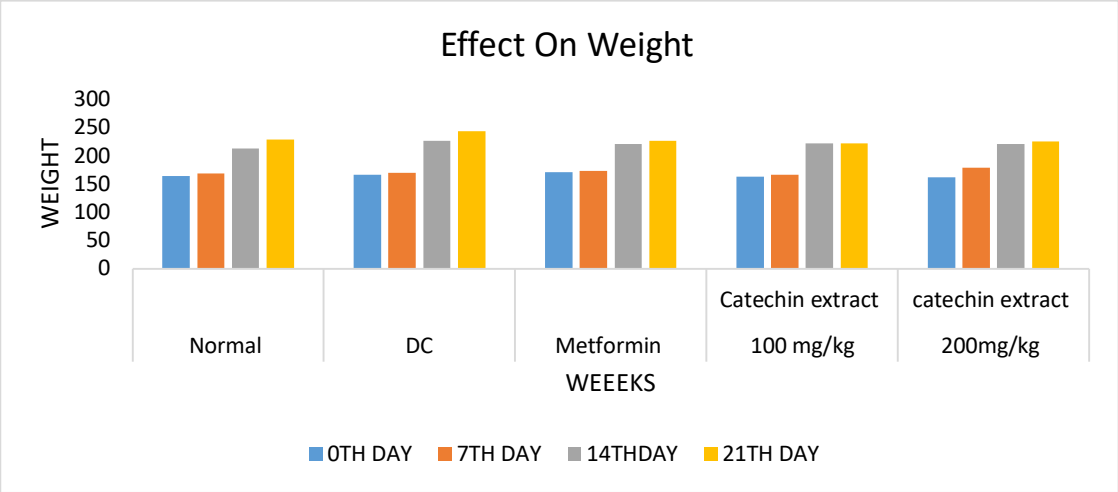

Figure VI: Effect of catechin extract on Weight After Every 7 Days

Table VI: Effect of catechin extract on post prandial blood glucose level

Days	NC	DC	Metformin	100mg/kg Catechin extract	200mg/kg Catechin extract
0th day	108.4	342	355	342.2	384.6
7th day	108	343.8	296.8	307.8	340
14th day	105.8	338	188.6	189.2	238.8
21st day	105.8	336	152	169.2	184.4

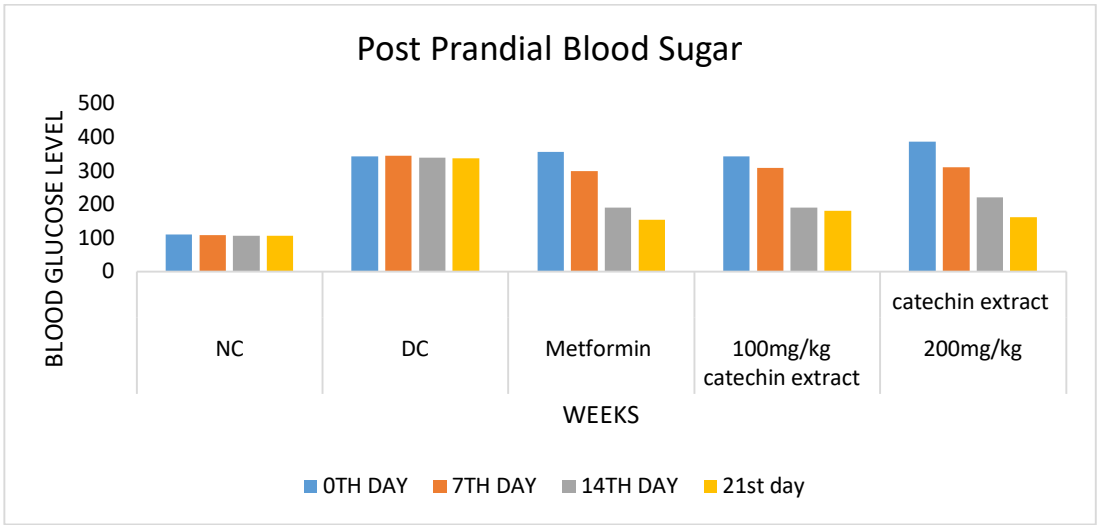

Figure VII: Effect of catechin extract on post prandial blood glucose level

Table VII: Effect of catechin extract on Liver Function Test

Sr No	NC	DC	Metformin	100mg/kg catechin extract	200mg/kg Catechin extract
SG0T	33.8	212.96	47.6	77	82.2
SGPT	36.8	28.6	30.6	28.2	22.8
Bilirubin	0.58	0.424	0.46	0.34	0.6
Total protein	6.9	28.6	8	10	8.76

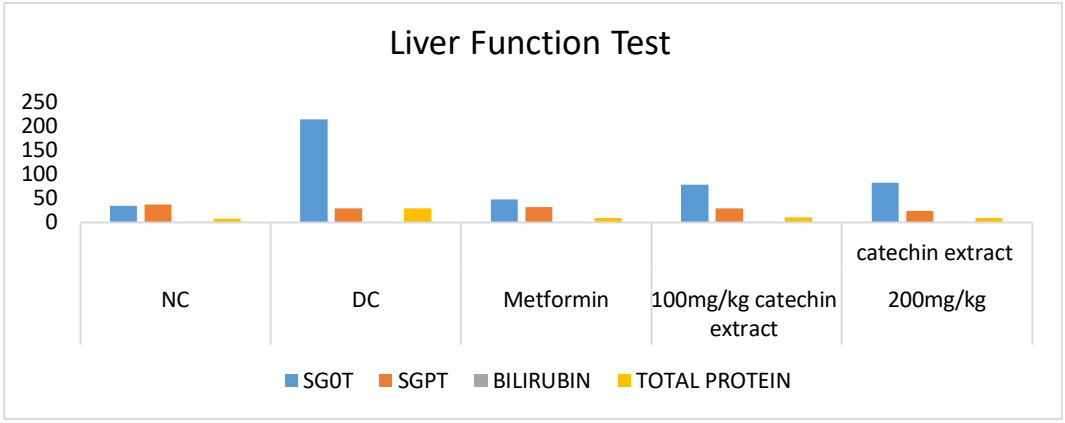

Figure VIII: Effect of catechin extract on liver function test

Table VIII: Effect of catechin extract on Kidney Function Test

Sr No	NC	DC	Metformin	100mg/kg catechin extract	200mg/kg Catechin extract
Creatinine	0.54	4.88	1.72	2.22	1.84
Bun	10	47.818	12.4	14.6	14.8
Bun/Creatinine ratio	10.6	93.732	14.2	16.994	19
Uric acid	1.76	1.418	1.718	1.64	1.68

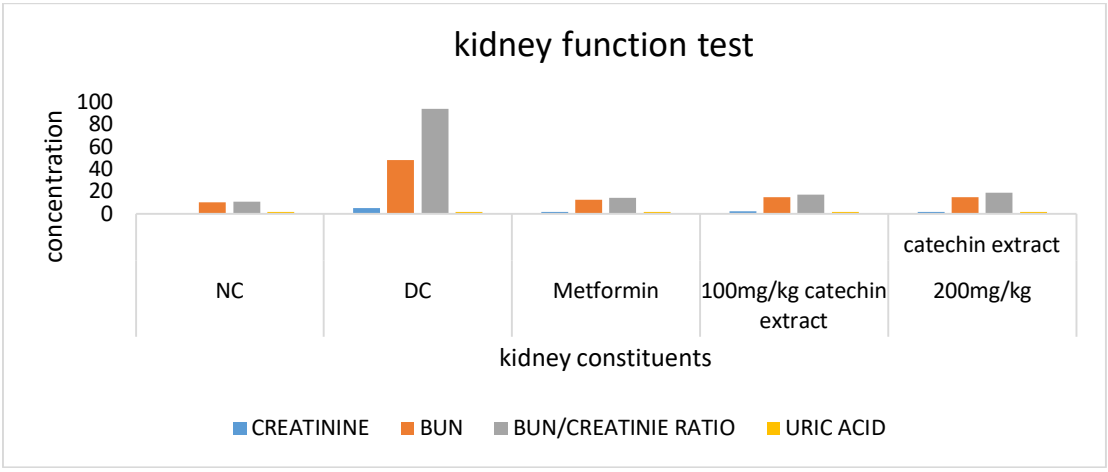

Figure XI: Effect of catechin extract on Kidney Function Test

Table XI: Effect of catechin extract on lipid profile

Sr No	NC	DC	100mg/kg catechin extract	200mg/kg catechin extract	Metformin 100mg/kg
Cholsterol	160.0	176	50.41	38	46
Triglyceride	240	236	55.8	49	51
LDL	166.1	200	52.89	42	59
VLDL	169.2	186	51.41	39	63
HDL	156.3	170	48	41.12	43

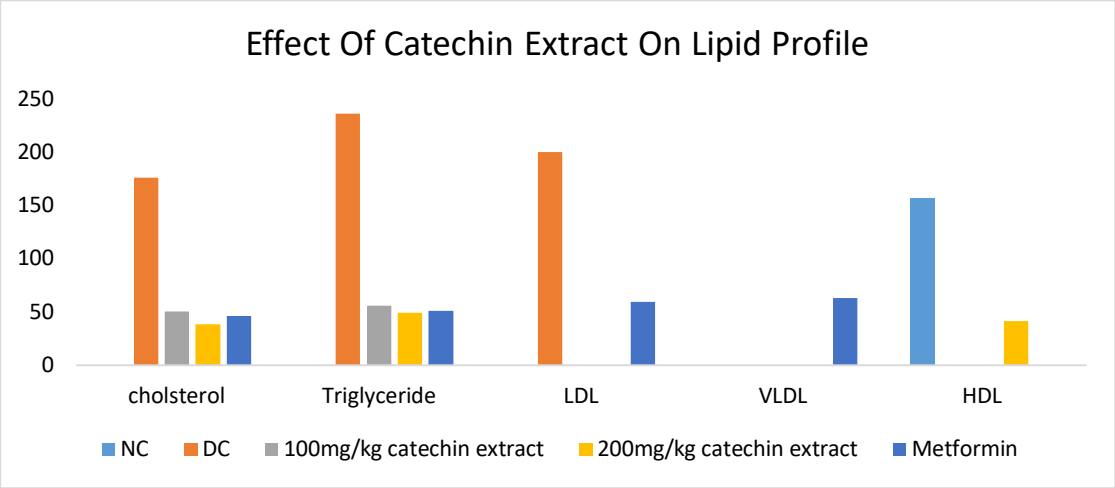

Figure X: Effect of catechin extract on lipid profile

DISCUSSION

Diabetes mellitus is a metabolic disorder where high blood glucose level leads to various chronic complications like neuropathy, retinopathy, nephropathy and various cardiovascular problems [15-19]. The major cause of diabetes is oxidative stress and for that antioxidants are require. There are numerous plants which acts as natural antioxidant. Synthetic medicines are present in wide quantity in market but with so many side effects. The side effects are not only due to API but also with excipients present in them. The side effects could be overcome by neutralizing any natural substitute. Green tea botanically known as camellia sinensis majorly used as a natural antioxidant drink [20]. Green tea consumption in high quantity leads to high acid secretion due to presence of caffein. Green tea possesses large number of gallocatechins, phenols and flavonoids which are mainly responsible for antioxidant activity. So that this study was under taken to isolate catechin extract from green tea. This study has shown catechin extract has high quantity of phenols and flavonoids. Total phenolic content and total flavonoid content has given high antioxidant activity which was confirmed by antioxidant assay i.e DPPH assay. This antioxidant activity was used for neutralizing metabolic disorder arising due to generation of free radicles. Diabetes mellitus is a metabolic disorder majorly due to generation of oxidative stress inside the body [21-24]. Thus catechin extract was investigated for in vitro and in vivo antidiabetic activity. In vitro and in vivo activity has shown good antidiabetic activity. When antidiabetic activity of catechin extract was compare with metformin it has given very good activity. Catechin extract if taken for further study would be a better alternative for synthetic drugs [25].

CONCLUSION

Catechin extract pronounced component of *camellia sinensis* was found to be a potent component for reducing blood glucose level. It also plays a vital role to reduce lipid profile, which protects from risk of cardiovascular diseases mainly coronary arterial disease. Hence it acts as an alternative or a helper medicine with metformin to reduce blood glucose level and chronic complications of type 2 diabetes.

CONFLICT OF INTEREST STATEMENT

We declare that we are not having any conflict of interest.

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