

## Evaluation of antidiabetic and antioxidative efficacy of ethyl acetate fraction of methanolic extract of *Camellia sinensis* (green tea) leaves in streptozotocin induced diabetic albino rat

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### Abstract

To investigate the antidiabetic and antioxidative potentiality of ethyl-acetate fraction of methanolic extract of *Camellia sinensis* (Green tea) leaves in streptozotocin induced diabetic rat. Streptozotocin induced diabetic state was confirmed by increased level of fasting blood glucose, decreased level of serum insulin along with inhibition in carbohydrate metabolomics. Oxidative stress was assessed by measuring antioxidative enzyme activities of hepatic and skeletal-muscular tissue. Hepatic *Hexokinase-I*, pro-apoptotic *Bax* and anti-apoptotic *Bcl-2* gene expression patterns were noted by qRT-PCR technique. After treatment with ethyl-acetate fraction of methanolic extract of *Camellia sinensis* (Green tea) leaves at a dose of 100 mg/kg body weight/day to diabetic rats for 28 days, a significant ( $p < 0.05$ ) recovery was noted in fasting blood glucose level, serum insulin level along with activities of carbohydrate metabolic enzymes in hepatic tissue in respect to the vehicle treated diabetic group. This fraction also resulted a significant ( $p < 0.05$ ) recovery in the activities of antioxidative enzymes in hepatic and skeletal-muscular tissue. In streptozotocin induced diabetic rat the low level of expression of *Hexokinase-I*, anti-apoptotic *Bcl-2* and high level of expression of pro-apoptotic *Bax* gene were observed in hepatic tissue in respect to vehicle treated control. There were recovered significantly after the treatment with the said fraction. From the results, it may be concluded that ethyl-acetate fraction of methanolic extract of leaves of *C. sinensis* has a promising anti-diabetic and antioxidative activities for the management of streptozotocin induced diabetic state.

**Keywords:** Streptozotocin, *C. sinensis*, Antioxidant, *Hexokinase-I*, *Bax*, *Bcl-2*

### Introduction

*Camellia sinensis* (*C. sinensis*) L. belongs to the Theaceae family has immense medicinal properties in the treatment and prevention of many diseases [1]. Diabetes mellitus (DM), a chronic metabolic disorder, now called syndrome is characterised by hyperglycaemia. This is mainly caused by insulin deficiency which is often combined with insulin resistance [2]. Diabetes and oxidative stress has a strong relationship between each other [3] Till now no effective medicine has been developed that offers a permanent cure in the management of diabetes and oxidative stress [4]. Recent decades have shown a resurgent interest in traditional plant medicine for the management of hyperglycaemia and oxidative stress [5]. The World Health Organization (WHO) also recommends that this practice should be encouraged especially in countries where access to

conventional treatment of diabetes mellitus is not adequate [6]. Moreover, an approach has been taken world wide to introduce "Food as Medicine". On that perspective the present study was designed to investigate the therapeutic potentiality of the ethyl acetate fraction of methanolic extract of *C. sinensis* (Green tea) leaves against hyperglycaemia and oxidative stress condition in experimental model diabetic rats.

### Materials and methods

#### Plant material preparation

*C. sinensis* (Green tea) leaves were used in this study. The leaves were purchased from Subodh Brothers Pvt. Ltd, Kolkata and preserved in air tight glass container.

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## Preparation of ethyl acetate fraction of methanolic extract of *C. sinensis* (Green tea) leaves

Leaves of *C. sinensis* of 100 g amount were suspended in 1 litre of methanol and kept at 25°C with intermittent stirring for first 2 h. After 24 hours, the mixture was filtered first using a cotton filter and then a Whatman filter paper (No. 1). The filtrate was dried under low pressure and the residue was collected. Ultimately 30-35 g of methanolic extract was prepared. In a 5 L separating flask, 35 g lyophilized extract of *C. sinensis* was dissolved in 500 ml methanol. Fractionation was carried out using polarity grade solvents i.e. n-hexane, chloroform and ethyl acetate. All these fractions were separated, collected and dried under reduced pressure at 40°C using rotavapor. The ethyl acetate fraction was dissolved in distilled water and administered orally to experimental diabetic rats for this experiment at a specific dose.

## Animal and animal care

Normoglycemic (fasting blood glucose level 70-80 mg/dl) wistar male albino rats having body weight about  $150 \pm 10$  g were used in this experiment. The animals were housed at a room temperature of  $25 \pm 2^\circ\text{C}$  under 12 hr : 12 hr light-dark cycle and acclimated to these conditions for 15 days before use in experiment. All animals were provided to standard rat feed and water *ad libitum*. The principles of laboratory animal care [7] and instructions given by our "Institutional Ethical Committee" (IEC/3/C-5/14 date: 3/11/14) were followed throughout the experiment.

## Induction of diabetes mellitus

Diabetes was induced to the rats as per our standardized method [8] and by other [9]. Rats, kept in fasting condition for 24 hours, were subjected to a single intramuscular injection of streptozotocin (STZ) (Sigma Chemical Co., USA) at a dose of 40 mg/ml of citrate buffer /kg body weight that produce diabetes (having fasting blood glucose level more than 250 mg/dl but less than 300 mg/dl) after 24h of STZ injection. This level of fasting blood glucose has been selected here as it represents the moderate diabetic state [10]. Subsequent six days were allowed for the stability of diabetes and after that the rats were selected for the experiment those were fulfilled the above criteria.

## Experimental design

To evaluate the antidiabetic effect of ethyl acetate fraction of methanolic extract of *C. sinensis* on STZ induced diabetes mellitus, all the rats were divided into 3 groups of 6 animals each. Except control group two other groups were made diabetic by single intramuscular injection of STZ at a dose of 40 mg/kg body weight. The duration of treatment were 28 days. On 29<sup>th</sup> day of treatment,

all the animals were sacrificed under light ether anaesthesia followed by decapitation at fasting state.

Group 1: Vehicle treated control group: Rats of this group were non-diabetic (normoglycemic) and were subjected to oral feeding of distilled water at the dose of 2 ml/kg body weight/rat for twice a day for 28 days.

Group 2: Vehicle treated diabetic group: Diabetic rats of this group were also treated with distilled water at the dose of 2 ml/kg body weight/rat for twice a day for 28 days.

Group 3: Fraction treated diabetic group: Diabetic rats of this group were treated with the ethyl acetate fraction of methanolic extract of *C. sinensis* (Green tea) leaves at 100 mg / kg body weight in 2 ml of distilled water/ for twice a day on and from 7<sup>th</sup> day STZ injection for next 28 days. Every day, in morning ( at 9.00 am) the first oral dose of the above said fraction was given 2 hour before supply of animal feed and in afternoon ( at 5.00 pm) the second dose was provided 2 hours after cleaning the feed box. After 2 hour of oral administration of the fraction, feeds were supplied to the animals. This protocol has been focused to minimise the drug-nutrient interaction if any.

On 29<sup>th</sup> day of fraction treatment, animals were sacrificed by decapitation under ether anaesthesia. Before decapitation, blood was collected from aorta and serum was prepared and used for serum insulin. Liver and skeletal muscle (quadriceps) were collected, washed in saline water, soaked in filter paper and preserved at  $-20^\circ\text{C}$  and then used for enzyme study.

## Measurement of hyperglycaemic bio-sensors

### Measurement of fasting blood glucose (FBG) level

At the time of grouping of the animals, FBG levels were measured. Levels of FBG were further recorded from all the animals of all groups in every seven days interval throughout the experimental period and on the day of animal sacrifice. Blood was collected from the tail vein by syringe following warm up the tail, within the experimental duration Fasting blood glucose level was measured using the single touch glucometer (Bayer's Ascensia Entrust, Bayer, Germany).

### Serum insulin level

Serum insulin level was measured by Rat Insulin ELISA Kit (Ray Biotech, Norcross, GA 30092 USA). Level of serum insulin was expressed in ng/dl.

### Assay of hexokinase activity

Hexokinase activities in liver and skeletal-muscular tissue were determined spectrophotometrically following the standard method [11]. The assay mixture contained 3.7 mM glucose, 7.5 mM  $\text{MgCl}_2$ , 11 mM thioglycerol, and 45 mM HEPES buffer. Tissues were

homogenized in ice-cold 0.1M phosphate-buffered saline (pH 7.4) to a concentration of 50 mg/mL. In a spectrophotometer cuvette, 0.9 ml of this assay mixture, 0.01mL glucose-6-phosphate dehydrogenase, 0.01 mL NADP and 0.03 ml of ATP were added and mixed well. After that, 0.6 mL of the tissue supernatant was added into the cuvette and absorbance was noted at 340 nm. One unit of hexokinase was expressed as  $\mu\text{g}$  /mg of tissue.

### Biochemical assay of glucose-6-phosphatase activity

Hepatic and skeletal muscle glucose-6-phosphatase activities were measured according to standard protocol [12]. Tissues were homogenized in ice cold of 0.1 M phosphate buffer saline (pH 7.4) at the tissue concentration of 50 mg/ml. In a calibrated centrifuge tube, 0.1 ml of 0.1 M glucose-6-phosphate solution and 0.3 ml of 0.5 M mallic acid buffer (pH 6.5) were taken and brought to 37°C in water bath for 15 min. The reaction was stopped with 1 ml of 10% trichloroacetic acid (TCA) followed by chilling in ice and centrifugation at 3000 *g* for 10 min. The optical density was noted at 340 nm. The enzyme activity was expressed as mg of inorganic phosphate liberated per g of tissue.

### Biochemical assay of glucose-6-phosphate dehydrogenase activity

Activities of glucose-6-phosphate dehydrogenase of liver and skeletal muscle were measured spectrophotometrically following the standard method [13]. Tissues were homogenized in ice cold 0.1 M phosphate buffer saline (pH-7.4) at the tissue concentration of 50 mg/ml. In a spectrophotometric cuvette, 0.3 ml of 1M Tris chloride buffer (pH-7.5), 0.3 ml of 2.5 10.2 M glucose-6-phosphate, 0.1 ml of 2 10.3 M NADP, and 0.3 ml of 0.2 M  $\text{MgCl}_2$  and 0.3 ml of ice cold tissue homogenate were taken. The rate of change of absorbance at 340 nm was recorded. One unit of enzyme activity define as that quantity which catalyses the reduction of  $1\mu\text{M}$  of NADP per minute.

### Assessment of oxidative stress biomarkers

#### Biochemical assay of catalase activity

Activities of catalase in hepatic and skeleto-muscular tissue were measured biochemically [14]. For the evaluation of catalase activity, liver tissues was homogenized in 0.05 M Tris-HCl buffer (pH-7.0) at the tissue concentration of 50 mg/ml. These homogenized samples was centrifuged at 10000 *g* at 4°C for 10 min. In spectrophotometer cuvette, 0.5 ml of 0.00035 M  $\text{H}_2\text{O}_2$  and 2.5 ml distilled water were added and mixed. Readings of absorbance was noted at 240 nm before the addition of supernatant. The supernatant was added at a volume of 40 $\mu\text{l}$  to the

cuvette and the kinetics was measured by recording successive six readings at 30 sec interval.

### Assessment of superoxide dismutase activity

Activities of superoxide dismutase in the hepatic and skeletal muscle tissue sample were estimated by measuring the percentage inhibition of the pyrogallol auto-oxidation by superoxide dismutase according to standard method [15]. In a spectrophotometric cuvette, 2.04 ml of 50 mM Tris buffer (pH-8.2), 20  $\mu\text{l}$  of sample and 20  $\mu\text{l}$  of pyrogallol were taken and the absorbance was observed in spectrophotometer at 420 nm for 3 min. One unit of superoxide-dismutase was defined as the enzyme activity that inhibits the auto-oxidation of pyrogallol by 50%.

### Histological study

The pancreatic tissue was dissected out and fixed in Bouin's fixative. Target tissue was subjected to paraffin embedding after dehydration process followed by tissue section cutting in rotary microtome (5  $\mu\text{m}$  thick). Sections were allowed to stain in heamatoxylin-eosin for microscopic observation. Qualitative analysis of islets histology was carried out on stained tissue from the view point of cell density and islets size.

### RNA extraction and cDNA synthesis

Animals were sacrificed and hepatic tissue was dissected out from the animal and the tissue was immediately allowed to freeze in liquid nitrogen. Then the frozen tissue was pulverized and resuspended in lysis buffer (Roche Diagnostics, Mannheim, Germany), RNA was extracted according to the manufacturers protocol. Residual DNA was removed by treatment with 5 units of DNase I (Roche Diagnostics, Mannheim, Germany) for 45 min at 37 C followed by inactivation for 10 min at 65 C. Then, 2  $\mu\text{g}$  of RNA was reversed transcribed with 'Superscript II reverse transcriptase'. The cDNA was diluted five-fold prior to PCR amplification.

### Real-time quantitative PCR

Real-time PCR was performed in the Light Cycler 480 (Roche Diagnostics, Mannheim, Germany) using the 'SYBR Green I Master mix' (Roche Diagnostics). Final mixture of PCR (final volume 20  $\mu\text{l}$ ) contained 10  $\mu\text{l}$  'SYBR Green I mix', 5  $\mu\text{l}$  of cDNA (1:5 dilution) and 0.5  $\mu\text{M}$  of each primer and 4  $\mu\text{l}$  PCR grade distilled water. Polymerase Chain Reaction(PCR) amplification was initiated with a 10-min pre incubation step at 95 C, followed by a 35 cycles of denaturation at 95 C for 10 s, annealing as described in table 2 and elongation at 72 C for 10 s. After amplification, the melting curves were determined in a three-segment cycle of 95 C for 5 s, 65 C for 15 s and 97 C for 0 s. All PCR reactions were performed in triplicates. Primer specificity was assessed through melting curve

analysis. Primers (rat specific) were designed for the gene of interest, i.e. *Hex-1*, *Bax*, *Bcl-2*, and glyceraldehyde 3-phosphate

dehydrogenase (GAPDH) as reference, the sequences of which given in detailed (Table -1)[16].

**Table 1:** Primer-sequence and specific conditions used for PCR amplification of candidate genes

Gene	Primer Sequence	Annealing temperature ( $^{\circ}$ C)	No. of Cycles	Amplification size (bp)
<i>Hex-1</i>	S 5'-GACCAAGTCAAAAAGATTGA-3' AS 5'-TCTTCTCGTGGTTCACCTGC-3'	62	35	256
<i>Bax</i>	S 5'-AGACAGGGGCTTTTGTAC-3' AS 5'-GAGGACTCCAGCCACAAAGAT-3'	58	35	482
<i>Bcl-2</i>	S 5'-CCGGGAGAACAGGGTATGAT-3' AS 5'-CAGGTATGCACCCAGAGTGA-3'	60	35	689
GAPDH	S 5'-ACCACAGTCCATGCCATCAC-3' AS 5'-TCCACCACCCTGTTGCTGTA-3'	58	35	452

GAPDH-glyceraldehyde 3-phosphate dehydrogenase, S- Sense, AS- Antisense, bp- base pair

### Assessment of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)

For the measurement of GOT and GPT activities, kits were used supplied by Crest Bio-systems, Gitanjali, Dr. Antonio Do RegoBagh, Alto Santacruz, Bambolim Complex (Goa, India) [17].

### Statistical analysis

All data were expressed in Mean  $\pm$  SEM, (n=6). For the statistical analysis of data, 'Analysis of Variance (ANOVA)' followed by multiple comparison two tail t-test was performed [18]. Differences were considered significant at  $p < 0.05$ .

## Results

### Carbohydrate metabolomics

#### Fasting blood glucose level

Fasting blood glucose was significantly ( $p < 0.05$ ) elevated in STZ-induced diabetic animals when compared with vehicle treated control rat. Treatment with said fraction resulted a significant ( $p < 0.05$ ) recovery in FBG (Table-2).

**Table 2:** Fasting blood glucose level correction by ethyl acetate fraction *C.sinensis* in diabetic model rat

Fasting blood glucose level (mg/dl)						
Groups	(on the day of STZ injection)	1 <sup>st</sup> day(The day of treatment started after 7days of injection)	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	29 <sup>th</sup> day
Vehicle treated control	74.33 $\pm$ 1.63 <sup>a</sup>	75.83 $\pm$ 3.31 <sup>a</sup>	76.5 $\pm$ 3.44 <sup>a</sup>	78.16 $\pm$ 3.1 <sup>a</sup>	73.83 $\pm$ 3.7 <sup>a</sup>	74.83 $\pm$ 3.12 <sup>a</sup>
Vehicle treated diabetic	76.66 $\pm$ 3.72 <sup>a</sup>	281.66 $\pm$ 3.14 <sup>b</sup>	275.3 $\pm$ 2.7 <sup>b</sup>	271 $\pm$ 3.22 <sup>b</sup>	276.3 $\pm$ 2.8 <sup>b</sup>	266. $\pm$ 3.57 <sup>b</sup>
Fraction treated Diabetic	77.3 $\pm$ 2.33 <sup>a</sup>	284.5 $\pm$ 3.44 <sup>b</sup>	256.1 $\pm$ 3.4 <sup>c</sup>	211.1 $\pm$ 3.9 <sup>c</sup>	174 $\pm$ 3.79 <sup>c</sup>	118.6 $\pm$ 3.20 <sup>c</sup>

Data represents Mean  $\pm$  SEM (n = 6). ANOVA followed by 'Multiple comparison two tail-t-test'. Values with different superscripts (a, b, c) in each vertical column differ from each other significantly,  $p < 0.05$ .

### Serum insulin level

A significant ( $p < 0.05$ ) decrease in the level of serum insulin was found in the STZ-treated diabetic group when compared with the

vehicle treated control group. The administration of the ethyl acetate fraction of said extract to the diabetic animals resulted a significant ( $p < 0.05$ ) recovery in the said parameter towards the vehicle treated control level (Figure. 1).

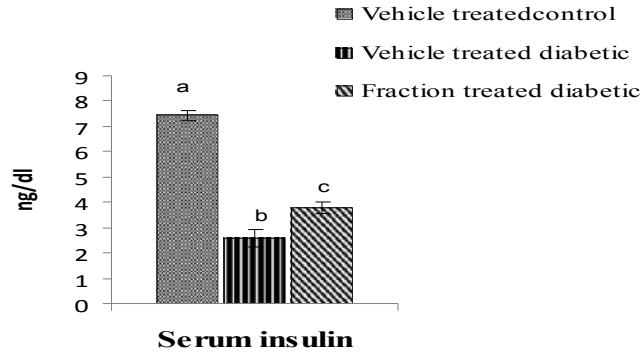
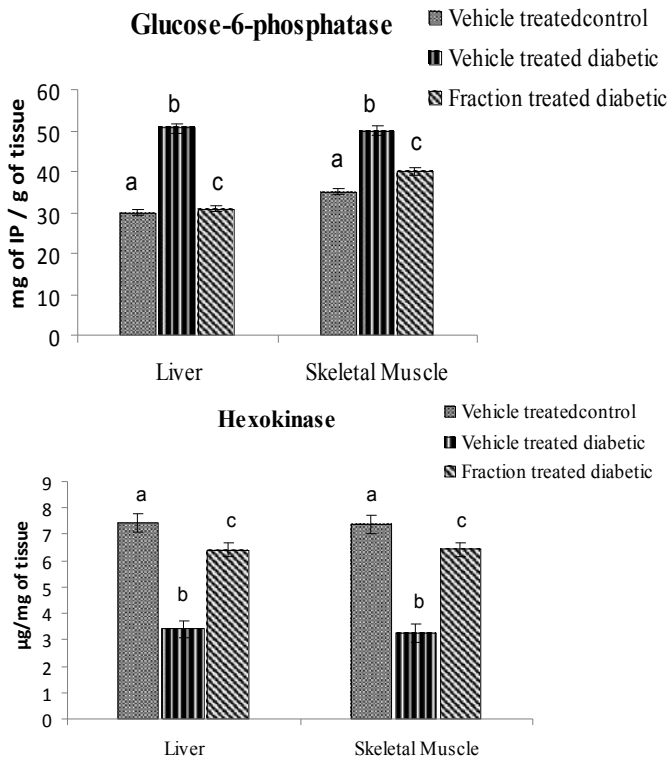


Figure 1: Effect of ethyl acetate fraction of *C.sinensis* on serum insulin level in diabetic model rat

### Hexokinase, glucose-6-phosphatase and glucose-6-phosphate dehydrogenase activities

Hepatic and skeletal muscle hexokinase and glucose-6-phosphate dehydrogenase enzyme activities were decreased significantly ( $p < 0.05$ ) accompanied by significant ( $p < 0.05$ ) increase in glucose-6-phosphatase activity in STZ-induced diabetic group when compared to the vehicle treated control group. After 28 days treatment with ethyl acetate fraction of methanolic extract of *C.sinensis* (Green tea) leaves to diabetic rats, significant ( $p < 0.05$ ) recovery was observed in the activities of these above enzymes (Figure. 2, 3 and 4).



### Glucose-6-phosphate dehydrogenase

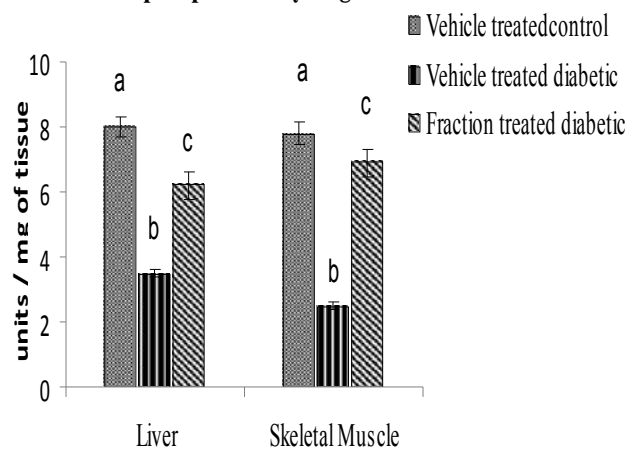


Figure 2, 3 and 4: Carbohydrate metabolic enzymes activities in ethylacetate fraction of *C. sinensis* treated in diabetic model rat. Data represents Mean  $\pm$  SEM (n = 6). ANOVA followed by 'Multiple comparison Student's two tail-t-test'. Bars with different superscripts (a, b, c) differ from each other significantly,  $p < 0.05$ .

### Oxidative stress markers profile

Catalase and superoxide dismutase enzyme activities in hepatic and skeletal muscle were significantly ( $p < 0.05$ ) decreased in diabetic group in respect to the vehicle treated control group. After the treatment with this ethyl acetate fraction of methanolic extract of *C.sinensis* (Green tea) leaves to the STZ-induced diabetic rat, the level of these parameters were restored significantly ( $p < 0.05$ ) towards the vehicle treated control (Figure. 5 and 6).



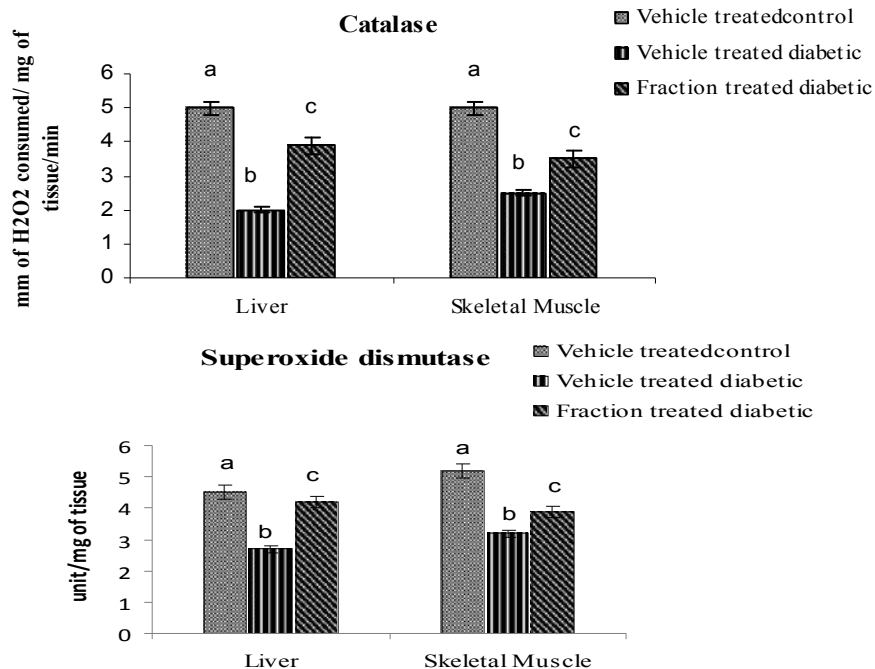


Figure 5 and 6: Antioxidant enzymes activities after treatment of ethyl acetate fraction of *C.sinensis* in diabetic model rat.

### Histological study

Cell population density and size of islets of Langerhans from qualitative point of view was significantly decreased ( $p < 0.05$ ) in

streptozotocin induced diabetic group in respect to the vehicle treated control group. Both were significantly restored towards the vehicle treated control group after ethyl acetate fraction of methanolic extract treatment in diabetic rat (Figure. 7).

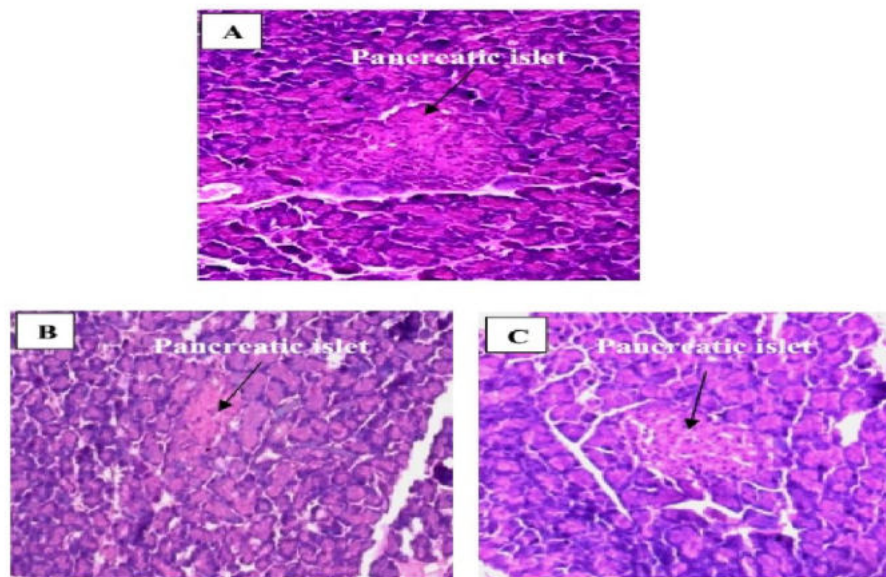
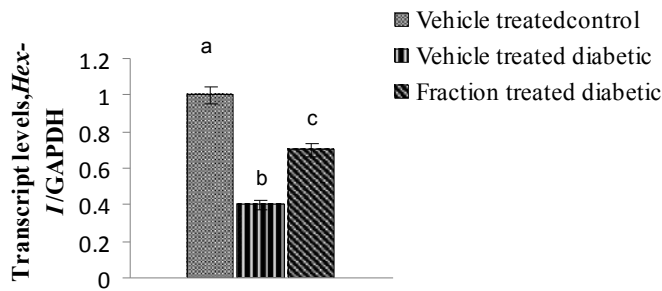


Figure 7: Microphotograph of pancreatic islets showing its correction after treatment of *C.sinensis* in diabetic model rat.

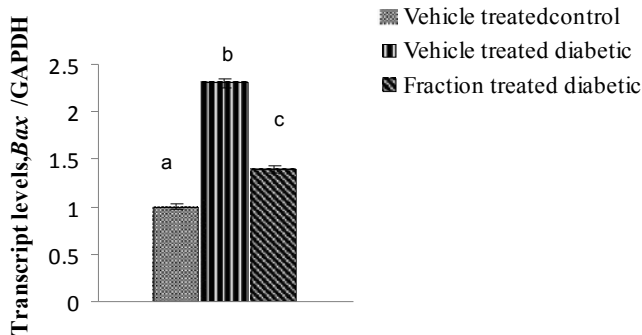


**Hex-I gene expression in hepatic tissue**

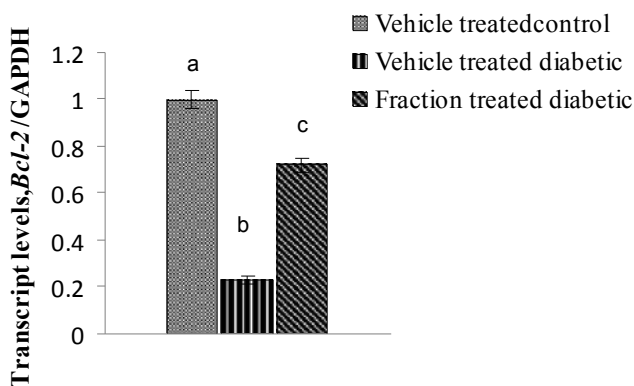


**Figure 8:** Hepatic *Hexokinase-I* gene expression in ethyl acetate fraction of *C. sinensis* treated diabetic model rat . Data represents Mean ± SEM (n = 6). ANOVA followed by 'Multiple comparison Student's two tail-t-test'. Bars with different superscripts (a, b, c) differ from each other significantly, p<0.05.

**Expression of Bax gene in hepatic tissue**



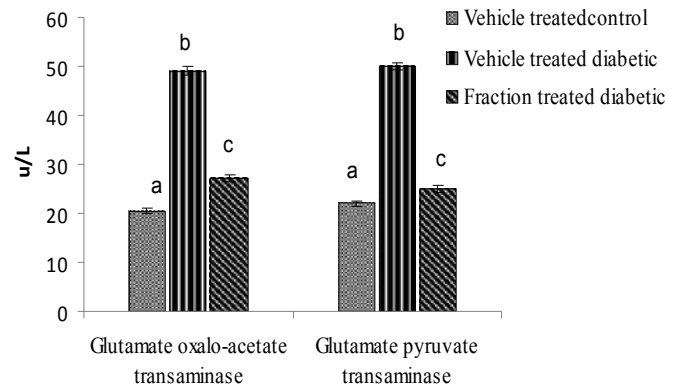
**Expression of Bcl-2 gene in hepatic tissue**



**Figure 9 and 10:** Hepatic *Bax* and *Bcl-2* gene expression in *C. sinensis* ethyl acetate fraction treated diabetic model rat. Data represents Mean ± SEM (n = 6). ANOVA followed by 'Multiple comparison two tail-t-test'. Bars with different superscripts (a, b, c) differ from each other significantly, p<0.05.

**Hexokinase-I gene expression pattern**

Hepatic *Hex-I* gene in STZ- induced diabetic rat showed a downward expression pattern which was noted by qRT-PCR analysis when compared with the vehicle treated control group. A significant (p<0.05) remedial effect of ethyl acetate fraction of methanolic extract of *C. sinensis* (Green tea) leaves was noticed in the said gene expression in target tissue after treatment to diabetic group (Figure. 8).



**Figure 11:** Hepatic GOT & GPT protection by *C. sinensis* of ethyl acetate fraction in diabetic model rat . Data represents Mean ± SEM (n = 6). ANOVA followed by 'Multiple comparisons Student's two tail-t-test'. Bars with different superscripts (a, b, c) differ from each other significantly, p<0.05.

**Expression of Bax, Bcl-2 genes**

Expression of *Bax* gene was increased and *Bcl-2* gene was decreased significantly (p < 0.05) in hepatic tissue in said STZ- induced diabetic rat with respect to the vehicle treated control group by qRT-PCR analysis. After the treatment of said fraction to diabetic group for 28 days, a significant recovery (p < 0.05) was observed in the expression pattern of hepatic *Bax* and *Bcl-2* genes towards the vehicle treated control group (Fig. 9 and 10).

**Toxicity study**

Activities of liver GOT, GPT were increased in STZ- induced diabetic group compared to the vehicle treated control group. A significant attenuation in the said enzyme activities towards the vehicle treated control level was found after administration of the said fraction of *C. sinensis* (Green tea) leaves to the vehicle treated diabetic group (Figure. 11).

**Discussion**



For ages, streptozotocin (STZ) has been used to induce a diabetic animal for research purpose. Mainly streptozotocin destroys the insulin secreting pancreatic  $\beta$ -cells by reactive oxygen species dependent oxidative damage and resulting in diabetes mellitus [19]. Consequently, there is a reduced secretion of insulin leading to clinical condition such as hyperglycaemic state [20]. Here an approach has been taken for correction of diabetic disorders by ethyl-acetate fraction of methanolic extract of *C.sinensis* because this fraction and extract both are most effective in this purpose which we have noted from our pilot work conducted using several solvents for extraction and fractionation. The *C. sinensis* has been considered of our interest as tea is the common beverage of our community. In this present study, the administration of ethyl-acetate fraction of methanolic extract of *C.sinensis* (Green tea) leaves effectively reduced the fasting blood glucose level in STZ induced diabetic rat. It may be implicated that the fraction may help in the regeneration of pancreatic  $\beta$ -cells which have been reported by others [21] as well as from our previous reports [22]. Moreover, this hypothesis has been strengthened here from the study of serum insulin level.

In streptozotocin induced hyperglycaemic state, decreased activities of hexokinase and glucose-6-phosphate dehydrogenase in liver as well as skeletal-muscular tissue along with an increased activity of glucose-6-phosphatase in the said tissue. After the supplementation of the said fraction of *C.sinensis* (Green tea) leaves, a significant recovery in the activities of key carbohydrate enzymes like hexokinase and glucose-6-phosphate dehydrogenase are under the positive control of insulin [2] along with the activity of glucose-6-phosphatase which is under the negative control of insulin [23] and these are in consistent with other's report in this line [24] as well as previous investigation from our laboratory [25]. This type of observation has been noted using other plant for this purpose in our laboratory [26].

During diabetes, hyperglycemia engenders free radicals and also impairs the endogenous antioxidant defense system in many ways. Antioxidant defense mechanisms involve both enzymatic and non-enzymatic strategies [27]. In this present study, oxidative stress bio-markers especially the activities of catalase and SOD were assessed. Decreased levels of catalase and SOD both in liver and skeletal muscle observed in diabetic rat in respect to the control group. Supplementation of this fraction resulted a significant restoration in the activities of antioxidant enzymes in diabetic rat which propose another mechanism of anti-oxidative effect of this said fraction in connection with the management of diabetes.

The histological study of islets of Langerhans with pancreatic acini further supported the above results where vehicle treated diabetic group showed degeneration of pancreatic islets cells, which was due to streptozotocin used in this study [28]. After administration of ethyl acetate fraction of methanolic extract of *C. sinensis* (green tea) leaves there was recovery in size of islets Langerhans .

To strengthened this observation based result, genomic study has also done here to show the remedial effect of this fraction in *Hexokinase-1* gene along with hepatic *Bax* and *Bcl-2* genes. The

physiological role of hexokinase which is the key carbohydrate metabolic enzyme of glycolysis has been studied by others [29] along with us [30]. A downward expression pattern of *Hex-1* was noted in STZ- induced diabetic rat but after administration of ethyl-acetate fraction an upward expression pattern of *Hex-1* was noted. From these result it may be indicated that the said fraction recovered the homeostasis of carbohydrate metabolism by influencing *Hex-1* gene expression through phytomolecule-gene interaction.

The present study also focussed that the pro-apoptotic gene *Bax* has expressed significantly at higher whereas anti-apoptotic gene *Bcl-2* at a lower level in diabetic rat. This imbalance between these two genes clearly state that in liver the involvement of reactive oxygen radicals has been suggested in apoptotic cell death of hepatocytes cells [31]. This condition were corrected near to the vehicle treated control in ethyl acetate fraction treated group. It may be predicted that the said fraction may contain some phytomolecule that balance the apoptosis of hepatic cells at the control level which is involved for glucose homeostasis as liver is main metabolic organ in this purpose.

The said fraction has no toxicity in general which has reflected from the study of GOT and GPT activities in hepatic tissue [32] which also focussed that the phytomolecules present in said fraction are free from hepato-toxicity.

## Conclusion

From the present study, it may be concluded that the common beverage i.e., tea contain some phytomolecule(s) which have a promising efficacy for the correction of most prevalent non communicable diseases in our community i.e., diabetes by modulating the gene expression of key enzymes of carbohydrate metabolism as well as  $\beta$ -cell regeneration. Side by side, it has some potent effect on hepatic protection in diabetes induced liver damage by regulating apoptosis. The investigation is going on for qualitative analysis of  $\beta$ -cell regeneration after fraction treatment by flow cytometric study which will clear the mechanism of action in this field in near future.

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## Conflict of interest

All the authors state that there are no conflicts of interest within this article.





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