ANTI-DIABETIC EFFECT OF POLYPHENOLS OF TAMARIND SEED COAT EXTRACTS AND ITS EXPERIMENTAL ANALYSIS ON STREPTOZOTOCIN INDUCED DIABETIC MICE

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ABSTRACT
To find out the effective concentration of polyphenols present in Tamarind Seed Coat Extract (TSCE) in treatment of diabetes as a nutraceutical compound. Tamarind seeds were obtained and finely powdered seed coats were prepared. Then dried seed coat extracts were prepared by ethanolic extraction and concentration under rotary vacuum evaporator. The dried extracts were then used to quantify the total polyphenols present in them. Antioxidant assays such as invitro DPPH and FRAP assays, Antidiabetic assays such as α-amylase, α-glucosidase and PTP1B inhibition assays and ACE inhibition assays were performed to find the effect of TSCE on these enzymes. Three different doses of TSCE were then orally administered in streptozotocin induced diabetic mice daily for 21 days. Glibenclamide was taken as a positive control. Finally, the blood glucose level was measured at 0th, 7th, 14th, 21st day in all the groups involved in the study. The histopathological analysis of tissue sections of pancreas and liver were also performed to evaluate the efficacy of TSCE in controlling Diabetes in mice. The total polyphenols of tamarind seed coat extracts were found to be 507.4 mg/g of Gallic Acid Equivalence. Further the antioxidant potential was estimated by invitro DPPH and FRAP assay which were found to be 71.2% and 71.71% respectively, while the in vitro antioxidant potential was estimated through α-amylase, α-glucosidase and PTP1B inhibition assays which were found to be 71.25%, 76.45% and 90.2% respectively. Additionally, the anti-hypertensive potential was carried out through ACE inhibition assay which was found to be 80.6%. The TSCE were found to control blood glucose level at the dose of 400 mg/KG B.W. at 21st day and the histopathological examinations of the same showed that TSCE were able to bring about a normal architecture to pancreatic and liver tissues comparable with the control. The TSCE is preclinically proven to possess significant anti-diabetic potential that has been established through the research work which could be developed as a nutraceutical supplement with further clinical analysis.

KEYWORDS: Tamarind Seed Coat Extracts, Polyphenols, Antioxidant activity, Anti-Hypertensive activity, In vitro Anti-Diabetic Analysis, Biochemical and Histological analysis.

1. INTRODUCTION
India has high prevalence of the diabetes with 41 million Indians having diabetes suggesting every fifth diabetic in the world is an Indian (Shashank et al., 2007). The increase in the number of people with diabetes observed worldwide and mainly in India is due to the trends in urbanization, population growth and physical inactivity. The number of cases of diabetes worldwide in 2000 among adult’s ≥20 years of age is estimated to be ~171 million (Sarah Wild et al., 2004). With the prevalence of diabetes in 2000s kept in mind the projections for the year 2030 are around 2-3 folds increase i.e., from 31.7 to 79.4 (in millions) (Sarah Wild et al., 2004). There are various classes of anti-diabetic medications available for the treatment which includes biguanides, dipeptidyl peptidases 4 (DPP-IV) inhibitors, Sodium glucose co-transporter (SGLT2) inhibitor, insulin, GLP1 agonists, SU, TZD. Though these anti-diabetic drugs are common in use in today’s world there are considerable adverse effects upon its use which have been reported by various research findings. Metformin, a derivative of biguanides, Vitamin B12 deficiency, which have been reported to cause anemia and neuropathy (risk in elderly) (Fogelman et al., 2016). Sitagliptin, Saxagliptin, Vidaagliptin, Linagliptin, Alogliptin are classes of DDP-IV inhibitors which cause pancreatitis and upper RTI infections as reported by Fogelman et al (2016). The use of SGLT2 inhibitors has been reported to Ketoacidosis (rare), Genital mycosis, May increase LDLc, Bone fractures (UKPDS, 1998). Thus these adverse side effects have led to the search for alternate medications.

With the alarming rates of increasing trends in diabetes in the country, the plants with high anti-oxidant and anti-diabetic properties are gaining attention due to their higher therapeutic value and low biological side effects. Amongst numerous phytochemicals, polyphenols are highly studies due to their high antioxidant activities (Nurhanani et al., 2015). Some polyphenols especially flavon-3-on (catechin type) has been reported to show both anticarcinogenic-proapoptotic and anti-mutagenic effects (Strick et al., 2000). Barjesteh et al. (2007) has reported that treatment of flavonoids have seen to cause increased DNA changes in cultured blood stem cells (Barjesteh et al., 2007). Thus polyphenols devoid of side effects are sought. Tamarindus indica L. (Tamarind), one of the most widespread trees of the Indian subcontinent is a large evergreen tree cultivated...
throughout India, except in the Himalayas and western dry regions. Tamarind is a multipurpose plant. The pulp of the fruit has been used as a spice in Asian cuisine, especially in the southern part of India, for a long time (Saideswara et al., 2012). The tamarind seeds are a by-product of the commercial utilization of the fruit. The seeds are also edible and are normally roasted and consumed as pulses. Commercially, tamarind seed is used as raw material for production of tamarind seed gum, for use in industries such as food and medicine, as thickening and stabilizing agent (Nurhanani et al., 2015). Studies carried out by Tsuda et al (1994), reveals that tamarind seed coat contains phenolic oxidants such as 2-hydroxy-3,0,4-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenyl acetate and epicatechin. Therefore, the main objective of the work is to optimize the effective concentration of polyphenols present in Tamarind Seed Coat Extract (TSCE) in preventing the onset of diabetes as a nutraceutical compound and mode of action of these polyphenols present in TSCE through in vitro and in vivo studies.

2. MATERIALS AND METHODS

2.1. Preparation of Tamarind seed coat powders

Ripened Tamarind fruit were purchased from the local market, after removal of the edible parts the tamarind seeds were then heated at 140°C, for 45 minutes in a hot air oven, cooled down to room temperature to crack the outer brown layer from kernels. Then the brown-red colored seed coats were processed to fine powder (Komuturina et al., 2004). The finely powdered seed coats were then extracted with ethanol through soxhlet extraction procedure. Before further processing, the extracts were concentrated under rotary vacuum evaporator at 40 °C and dried to obtain a dry powder of crude polyphenol extract free from the organic solvent i.e., ethanol.

2.2. Analysis of total polyphenolic content

The polyphenol content of tamarind seed coasts was determined as demonstrated by Singleton et al (1975), Polyphenolic content of the fractions was assessed using the Folin-Ciocalteu assay, by employing Gallic acid standard. Equal volumes of extracts and Folin-Ciocalteu reagent (1N) were added and incubated at 37°C for 5 minutes followed by addition of 3 mL of 7% Sodium carbonate (Na2CO3). Then it was incubated at room temperature for 10 minutes. The absorbance was taken at 760 nm and recorded for further evaluation.

2.3. DPPH Radical Scavenging activity

The free radical scavenging activity of the phenolic extracts were determined as described by Brand-Williams et al (1995) with ascorbic acid as reference compound. To 0.1 mL of varying concentrations of phenolic extract, 3.9 mL of DPPH solution (2.4 mg in 100 mL of methanol) was added. The mixture was shaken vigorously and incubated at room temperature for 30 minutes in dark. The absorbance was measured at 515 nm using a spectrophotometer. The percentage radical scavenging activity was then calculated by diminishing color of the reagent.

2.4. Ferric reducing antioxidant potential assay

The ferric reducing antioxidant potential assay was performed as described by Vijayalakshmi et al (2016), 50 µL of varying concentrations of phenolic extracts were added to 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide solution. The reaction mixture was then mixed well and incubated at 50°C for 20 minutes in a vortex shaker. 2.5 mL of 10% (w/v) trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant was then mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% (w/v) ferric chloride (FeCl3). The absorbance was measured using spectrophotometer at 700 nm against blank with reference standard as ascorbic acid.

2.5. In Vitro α-Amylase Inhibition Assay

In vitro α-amylase inhibition assay was carried out based on the spectrophotometric assay using acarbose as the reference compound (Bhutkar et al., 2012). The modified DNSA method was carried out in which varying concentrations of TSCE extracts were pre-incubated with 1mL of 1 unit/mL α-amylase solution for 30 minutes at room temperature followed by addition of 1 mL of (1% w/v) starch solution which was then further incubated at 37°C for 30 minutes. The reaction was finally stopped with the addition of 1 mL of DNS reagent and the contents were incubated in a water bath for 5 mins. A blank was prepared one without seed coat extracts and another without amylase enzyme replaced by equal quantities of buffer (20 mM Sodium phosphate buffer with 6.7 mM Sodium chloride, pH 6.9 at 20°C). The absorbance was measured at 540 nm. The inhibition activity of α-amylase was used to determine the anti-diabetic activity using the following formula:

\[
\% \text{ Inhibition} = \left[1 - \frac{\text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}} \right] \times 100
\]

2.6. In vitro α-Glucosidase Inhibition Assay

The assay was performed as described by Apostolidis et al (2007). To varying concentrations of phenolic extracts 100µL of α-Glucosidase solution (1 U/mL) in 100 mM of phosphate buffer (pH 6.9) was added and incubated at 25°C for 10 minutes. Then, 50 µL of 5 mM p-nitrophenyl-d-glucopyranoside solution in 100 mM phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25°C for 5 minutes and the absorbance readings were taken at 405 nm in the spectrophotometer. The inhibitory activity was measured as percentage inhibition using the following formula:

\[
\% \text{ Inhibition} = \left[1 - \frac{\text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}} \right] \times 100
\]

2.7. Protein Tyrosine Phosphatase 1B Inhibition Assay

The assay was carried out using 96 well clear polystyrene microtiter plate according as described by Baumgartner et al (2010) with slight modifications. The activity was determined using 2mM PNPP as a substrate. 100 µM sodium orthovanadate was used as a positive control. The fractions were dissolved in 1% DMSO solution as it showed no influence in the assay. The activity was measured in the presence of 1mM PTP1B. After 30min incubation the reaction was stopped with 10M NaOH solution. The absorbance was measured at 405nm with a
photometer. The percent residual activity was calculated using the following formula:
Residual Activity (%) = (Abs_{FE} - Abs_{TS} - Abs_{Cur}/Abs_{FE} - Abs_{Cur}) × 100

Where, Abs_{FE} was the absorbance of p-nitrophenol liberated by the enzyme in the system without a test sample in contrast to Abs_{TS} (with enzyme sample).

2.8. Angiotensin converting enzyme Inhibition Assay
The assay was performed with hippuryl-L-histidyl-L-leucine (HHL) as substrate. The enzyme solution was preincubated with varying concentrations of the inhibitors for 20 minutes at 37°C in a 50 µL volume of sodium borate buffer (0.4M borate, 0.3M NaCl, pH 8.3). 10 µL of HHL substrate was added to the 25 µL preincubated solution followed by incubation at 37°C for 60 minutes. The reaction was stopped with 150µL NaOH solution followed by incubation at room temperature with addition of 20µL (20mg/mL) o-phthalaldehyde (OPA). The reaction was stopped with the addition of 50µl 1 M HCl and read at fluorescent plate reader at 355nm excitation and 535nm emission wavelength.

2.9. In vivo experimental animal studies
The in vivo experimental study was conducted on Wistar strain male albino mice. 3 months of age weighing about 45±5 g. Prior to the experiment, the animals were acclimatized for a period of 15 days in laboratory conditions. Mice were housed in colony cages (6 mice per cage), at an ambient temperature of 25±2 °C with 12 hour light: 12 hour dark cycle. Mice had free access to standard food and water ad libitum. The Principles of Laboratory Animal Care (CPCSEA Registration number – Protocol 7/2017: 1398/PO/Re/S/10/CPCSEA; Dated: 26-04-2017) is followed throughout the duration of experiment and instruction given by Institutional Ethical Committee (IAEC-SVCE) was followed regarding injection and other treatment of the experiment. Normo-glycemic animals was selected for this experiment having the fasting blood glucose level of 75±5 mg/dl.

2.10. Induction of Diabetes Mellitus
Mice were fasted for 24 h before the induction of diabetes by STZ injection. Experimentally induced diabetic condition was developed in a separate group of male Wistar strain albino mice by single intraperitoneal injection of STZ at the dose of 150 mg / 0.5 ml of citrate buffer / kg body weight / mice for five consecutive days (Maiti et al., 2005). This dose of STZ produce type-II diabetes mellitus having fasting blood glucose level more than 120 mg/dL to 250 mg/dL, after 24 h of STZ injection and this diabetic state was maintained throughout the experimental schedule (Maiti et al., 2005).

2.11. Experimental Design for in vivo study
Forty two mice were divided into seven groups. Each group had six mice for experimental process. The group is as given below:
• Control group: Mice of this group were made to receive intraperitoneal injection of citrate buffer (0.5 ml/ kg body weight/mice).
• Diabetic group: The mice were made diabetic by intraperitoneal injection of STZ (150 mg/0.5 ml citrate buffer / kg body weight/mice).

• Positive control group: Streptozotocin-induced diabetic rats was treated with Glibenclamide (DG) (2.5 mg/kg BW) for 21 days to compare with the TSCE polyphenol
• Diabetic + Tamarind seed coat extract supplemented group: The Diabetic mice was fed aqueous extract of seed of T. indica at varying doses of 200 mg (6 mice for 21 days), 300mg (6 mice for 21 days), 400mg (6 mice for 21 days) /0.5 ml of citrate buffer/ kg body weight/day/mice.
• Optimized dose experimental group: This group containing a set of six mice was used for further analyses to prove the antidiabetic effect of TSCE in diabetes induced mice.

Animals of control group, diabetic groups and the treatment groups were subjected to be feeding of 0.5 ml citrate buffer/100 g body weight/d for 21 days to keep all the animals at same type of treatment condition in respect to TSCE supplemented groups.

Estimation of Blood Glucose Level
In the experimental group of the animals with the time interval of 0th, 7th, 14th, 21st days of supplementation of TSCE blood was collected from tip of the tail vein and blood glucose level was measured using glucometer (Suzanne et al., 1991). The results were expressed in term of mg/dl of blood.

2.12. HISTOPATHOLOGICAL ANALYSIS
Further to confirm the anti-diabetic potential of TSCE histopathological analysis on liver and pancreas of experimental mice was carried out as per the guidelines of Hsa et al., (1981).

3. STATISTICAL ANALYSIS
The results of statistical analysis were expressed as Mean ± Standard Error Mean (SEM). One-way ANOVA and post-ANOVA (Tukey’s post hoc test) were used to compare the means of untreated group of normal mice with diabetic groups of mice treated as well as conventional drug treated diabetic mice and plant extract treated diabetic mice at various dosages.

4. RESULTS
4.1. Preparation of Tamarind Seed Coat Powder and Its Total Polyphenol content
Upon extraction and filtration (with Whatman No. 1 filter paper), the extracts were observed to be brown red in color which shows that they have a potentially rich sources of polyphenols as shown in Fig 1 and 2. Further quantifications of total polyphenolic contents with gallic acid reference standards resulted with the identification of concentration of the polyphenols of tamarind seed coat extract as 507.4 mg/g (Gallic acid equivalence) as shown in Fig 3.

4.2. DPPH assay
The Fig 4, shows that the free radical scavenging activity of the tamarind seed coat extracts on DPPH radicals increased with concentrations of polyphenols. Thus at concentration between 50-200 µg/mL the free radical scavenging activity increased with the dose dependent manner. At high concentration (250 µg/mL) of TSCE the DPPH scavenging activity was seen high (71.2%). The obtained result was comparable with the reference standard ascorbic acid (88%).
Day treatment

powdered form of Tamarind

In vivo

compared with control.

α%

-%

%.

3:

α of Tamarind Seed coat extracts

FRAP assay

potential antioxidant powers.

st

Tamarind seeds

% at 500 μg/mL for TSCE extracts while

Glucosidase and Protein Tyrosine

Glucosidase

Amylase, α-

st
e values was seen

FRAP assay

% as

Powder obt

study

2

Tamarind seeds

Glucosidase

- study

In vivo
tage of inhibition was

percentage inhibition was observed at 250 μg/mL

Glucosidase and Protein Tyrosine

The Total Polyphenol Assay

% at 500 μg/mL for TSCE extracts while

FRAP assay

% -

powdered form of Tamarind

- study

2

Tamarind seeds

Glucosidase

- study

in this current study.

was equivalent to the activity of oral drug Glibenclamide

4.3. FRAP assay

Fig 5, shows the ferric reducing antioxidant potential of TSCE in which the percentage antioxidant potential was seen as 71.71% at 500 g/mL for TSCE extracts while 92.67% at 500 g/mL. Such potential antioxidant powers may due to the hydroxyl benzene derivatives and epicatechin present in the seed coats.

4.4. α-Amylase, α-Glucosidase and Protein Tyrosine Phosphatase 1B inhibitory assay

The α-Amylase inhibitory activity and α-Glucosidase inhibitory activity of the tamarind seed coat extracts was seen to increase in a dose dependent manner. The maximum inhibitory activity towards amylase was found to be 71.20% and glucosidase was found to be 76.45% as shown in Fig 6 & 7, respectively. These values were seen lower than the reference standard acarbose which was observed to have a maximum inhibitory effect of 88±1.89%. Fig 8 shows the inhibitory activity of TSCE on PTP1B enzyme and was found to increase in a dose dependent manner and the percentage of inhibition was found to be 90.2%.

4.5. Angiotensin Converting Enzyme inhibition assay

Fig 9 shows the inhibitory activity of TSCE on ACE. The inhibitory activity was found to increase in a dose dependent manner from 50 g/mL to 250 g/mL. The highest percentage inhibition was observed at 250 g/mL and it was found to be 80.6%.

4.6. In vivo study

Data represented by Fig. 10 & Table 1 shows that TSCE at concentration of 400 mg/kg was found to reduce the blood glucose to 98±1.851 mg/dL on 21st day when compared with the lower concentrations of TSCE on different days. This optimal concentration of TSCE on 21st Day treatment was equivalent to the activity of oral drug Glibenclamide in this current study.
FIGURE 4: The DPPH Radical Scavenging Activity of TSCE
The values are mean + SEM from n=3 in Triplicates.
**Significant at p<0.01, compared with control.

FIGURE 5: The Ferric reducing antioxidant power assay of TSCE
The values are mean + SEM from n=3 in Triplicates.
**Significant at p<0.01, compared with control.

FIGURE 6: α-Amylase Inhibitory Assay of TSCE
The values are mean + SEM from n=3 in Triplicates.
**Significant at p<0.01, compared with control.

FIGURE 7: α-Glucosidase Inhibitory Assay of TSCE
The values are mean + SEM from n=3 in Triplicates.
**Significant at p<0.01, compared with control.

FIGURE 8: PTP1B Inhibitory activity Assay of TSCE
The values are mean + SEM from n=3 in Triplicates.
*Significant at p<0.05.

FIGURE 9: The Angiotensin converting enzyme Inhibitory assay of TSCE.
The values are mean + SEM from n=3 in Triplicates.
*Significant at p<0.05.
The effect of polyphenols of Tamarind seed coat extract on Blood Glucose levels (mg/dL) in STZ induced type 2 diabetic mice. C: Glibenclamide treated mice (2 mg/kg body weight), D: TSCE - treated diabetic mice (200 mg/kg body weight), E: TSCE - treated diabetic mice (300 mg/kg body weight) and F: TSCE - treated diabetic mice (400 mg/kg body weight). Arrows indicate islets of Langerhans. H & E staining, 40x.

**TABLE 1:** The effect of polyphenols of Tamarind seed coat extracts Blood Glucose levels (mg/dL)

<table>
<thead>
<tr>
<th>Day</th>
<th>Normal Mice*</th>
<th>Diabetic Control†</th>
<th>Group 3 (200 mg/Kg)‡</th>
<th>Group 4 (300 mg/Kg)§</th>
<th>Group 5 (400 mg/Kg)¶</th>
<th>Glibenclamide ¶*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>91±1.2293</td>
<td>208±1.6073**</td>
<td>207±0.7601***</td>
<td>214±2.3202**</td>
<td>211±1.859**</td>
<td>210±0.918</td>
</tr>
<tr>
<td>7</td>
<td>93±1.7272</td>
<td>212±1.6533**</td>
<td>176±1.2494**</td>
<td>159±1.3904**</td>
<td>144±1.447**</td>
<td>116±3.961</td>
</tr>
<tr>
<td>14</td>
<td>92.5±1.7272</td>
<td>212±1.6533**</td>
<td>152±0.7923**</td>
<td>141±1.4757**</td>
<td>120±1.681**</td>
<td>107±3.040</td>
</tr>
<tr>
<td>21</td>
<td>92.5±1.7272</td>
<td>212±1.6533**</td>
<td>120±1.6816**</td>
<td>110±2.6289**</td>
<td>98±1.851**</td>
<td>93±2.155</td>
</tr>
</tbody>
</table>

*: Significance between each pairs. **The Values are significant at the p<0.01. *** The Values are significant at the p<0.001. *The Values are significant at the p<0.05, NS – Non Significant by using Post Hoc Tukey HSD Test.

**FIGURE 10:** Determination of the effect of TSCE on Blood Glucose levels on STZ induced Diabetic mice (experimental)
The values are mean ± SEM from n=6. Significant at *p<0.05, **p<0.01, ***p<0.001. The statistical analysis was carried out using Post Hoc Tukey HSD Test.

**FIGURE 11:** Histopathology of pancreatic tissue
A: Normal Mice, B: Untreated streptozotocin induced type 2 diabetic mice, C: Glibenclamide treated mice (2 mg/kg body weight), D: TSCE - treated diabetic mice (200 mg/kg body weight), E: TSCE - treated diabetic mice (300 mg/kg body weight) and F: TSCE - treated diabetic mice (400 mg/kg body weight). Arrows indicate islets of Langerhans. H & E staining, 40x.
PTP1B inhibitory activity observed in this study were hypertensive Sargassums treated diabetic mice (300 mg/kg body weight) and F: TSCE et al (2006), ACE 12:

**DISCUSSION**

The FRAP assay data was comparable to those reported for Glucosidase when compared with (200 mg/kg body weight). The inhibitory assay activity reported in the present study is in accordance with the result reported by Meltem et al (2012) who studied the inhibitory activity of α-Amylase, α-Glucosidase on tea and grape seed extracts. They have also reported that the grape seeds were potent inhibitors of both the enzymes and tea polyphenols were potential inhibitors of α-Glucosidase when compared with acarbose reference standard. The PTP1B inhibitory activity observed in this study were comparable with the PTP1B inhibitory activity of Plastoquinones from Marine Brown Alga Sargassums erratifolium as reported by Yousof et al (2017). Higher values reported in this study suggest that the higher concentrations of extracts are required to accomplish the desired inhibitory activity as reported by Yousof et al (2017). Since PTP1B inhibitors are considered as a negative regulator of insulin receptor (IR) signaling, they could act as a potential drug in treating diabetes. Comparing these results in this study for ACE inhibition with the study carried out by Kwon et al (2006), ACE inhibition by TSCE was higher than that of oregano (30.4%) and similar with lemon balm (81.9%) while lower than that of Rosemary LA (90.5%). Siddesha et al (2010) reported that the T. Indica seed coat extracts shows good ACE inhibition of ethanolic, methanolic and aqueous extracts that acetonc extracts in addition to their suggestion to the benefit of polar compounds as potent ACE inhibitors. Thus, TSCE also exhibits excellent potential in ACE inhibition hence it could be used as a potential anti-hypertensive compound in addition to its use in diabetes.

The invivo study on blood glucose level in TSCE administered experimental animal was comparable with the previous studies reported by Sivashanmugam et al (2013) in which they observed that the treatment with the polyphenols on diabetic rats resulted in a significant decrease in blood glucose levels from the first weeks onwards. The mechanism of glycemic control of TSCE could be due to the insulin mimetic effects as reported by Daisy et al (2009). Thus a speculation can be made to justify the antidiabetic action of TSCE could be due to the synergic actions of various polyphenols present in the tamarind seeds. In the present study, histopathological evaluation of the pancreas, revealed a high frequency of multifocal necrosis of hepatocytes. The trend observed in the current study clearly indicates that for the current experimental period the optimal dosage of the tamarind seed coat extracts (400 mg/kg BW) reduced the necrosis brought back the cellular arrangements to normal limits. While diabetic mice treated with the tamarind seed coat extracts (200 mg/kg BW) shows fatty infiltrations with mixed, minimal and multifocal necrosis of hepatocytes, diabetic mice treated with the tamarind seed coat extracts (300 mg/kg BW) shows fatty infiltrations with mononuclear, minimal and multifocal necrosis of hepatocytes. The trend observed in the current study clearly indicates that for the current experimental period the optimal dosage of the tamarind seed coat extracts is 400 mg/kg BW to bring back the hepatocytes to their normal cell architecture. Liver section of Glibenclamide treated diabetic mice maintained the architecture within normal limits.

4.7. Histopathological analysis

Fig 11 shows the Histopathology of Pancreatic tissue section in the control mice showed normal histology. In diabetic control mice, the pancreatic section showed atrophy and degeneration of β cell islets. The islet cells were seen to be shrunken in size compared to the normal control and small. The pancreatic section of diabetic mice treated with the tamarind seed coat extracts (400 mg/kg BW) showed β cell regeneration comparable to that observed in Glibenclamide treated diabetic mice, while the sections of the tamarind seed coat extracts (200 mg/kg BW and 300 mg/kg BW) showed atrophy with mild regeneration of β cell islets.

Fig 12 shows the Histopathology of liver tissue section in the control liver tissue section showed normal architecture and morphological changes within the limits. Whereas the diabetic mice liver tissue section showed the distortions in the arrangement of cells and fatty infiltrations with mixed, minimal and multifocal necrosis of hepatocytes. Diabetic mice treated with the tamarind seed coat extracts (400 mg/kg BW) reduced the necrosis brought back the cellular arrangements to normal limits. While diabetic mice treated with the tamarind seed coat extracts (200 mg/kg BW) shows fatty infiltrations with mixed, minimal and multifocal necrosis of hepatocytes, diabetic mice treated with the tamarind seed coat extracts (300 mg/kg BW) shows fatty infiltrations with mononuclear, minimal and multifocal necrosis of hepatocytes. The trend observed in the current study clearly indicates that for the current experimental period the optimal dosage of the tamarind seed coat extracts is 400 mg/kg BW to bring back the hepatocytes to their normal cell architecture. Liver section of Glibenclamide treated diabetic mice maintained the architecture within normal limits.

5. DISCUSSION

In this study the TSCE were examined for its hypoglycemic activity, anti-diabetic and anti-hypertensive activity. Higher concentrations of phenolics were observed in the present study which was comparable to those values as reported by Loreth et al (2009) which was 713.24 mg/100g GAE with 95% EtOH as a solvent. The Phenolics content of TSCE was found to be higher than that of Tamarind fruit pulp as reported by Lamien-Meda et al (2008).

TSCE have strong antioxidant potentials which were evident through the DPPH and FRAP assays. The effects of DPPH radical scavenging activity observed in this study was in coherent those data observed by Sandesh et al (2014). The FRAP assay data was comparable to those results reported by Vijayalakshmi et al (2016). The increase in antioxidant activity in a dose dependent manner observed in DPPH and FRAP assays were coherent with the study reported by Siddharaju et al (2006). The inhibitory assay activity reported in the present study is in accordance with the result reported by Meltem et al (2012) who studied the inhibitory activity of α-Amyle, α-Glucosidase on tea and grape seed extracts. They have also reported that the grape seeds were potent inhibitors of both the enzymes and tea polyphenols were potential inhibitors of α-Glucosidase when compared with acarbose reference standard.

The PTP1B inhibitory activity observed in this study were comparable with the PTP1B inhibitory activity of Plastoquinones from Marine Brown Alga Sargassums erratifolium as reported by Yousof et al (2017). Higher values reported in this study suggest that the higher concentrations of extracts are required to accomplish the desired inhibitory activity as reported by Yousof et al (2017). Since PTP1B inhibitors are considered as a negative regulator of insulin receptor (IR) signaling, they could act as a potential drug in treating diabetes. Comparing these results in this study for ACE inhibition with the study carried out by Kwon et al (2006), ACE inhibition by TSCE was higher than that of oregano (30.4%) and similar with lemon balm (81.9%) while lower than that of Rosemary LA (90.5%). Siddesha et al (2010) reported that the T. Indica seed coat extracts shows good ACE inhibition of ethanolic, methanolic and aqueous extracts that acetonc extracts in addition to their suggestion to the benefit of polar compounds as potent ACE inhibitors. Thus, TSCE also exhibits excellent potential in ACE inhibition hence it could be used as a potential anti-hypertensive compound in addition to its use in diabetes.

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**FIGURE 12: Histopathology of liver tissue**

A: Normal Mice, B: Untreated streptozotocin induced type 2 diabetic mice, C: Glibenclamide treated mice (2 mg/kg body weight), D: TSCE - treated diabetic mice (200 mg/kg body weight), E: TSCE - treated diabetic mice (300 mg/kg body weight) and F: TSCE - treated diabetic mice (400 mg/kg body weight). H & E staining. 40x.
regenerative changes in the islets of diabetic mice. The neogenesis of β-cells could be due to the mechanism reported by Sole et al., (2012) in which they revealed the significant reduction in apoptosis by the action of Tamarind Seed extracts.

6. CONCLUSION
The current study proves that the tamarind seed coat extract (TSCE) contains highest concentration of polyphenols. The studies also showed the potent antioxidant activity of TSCE through DPPH radical scavenging activity and FRAP assays. The *in vitro* anti-diabetic effect of TSCE also showed the excellent glucose reducing effect through alpha amylase, alpha Glucosidase and protein tyrosine Phosphatase 1B inhibition assay studies. In addition, TSCE also showed a potent anti-hypertensive activity there by showing its ability in controlling the blood pressure and blood glucose level reducing effect. Plasma blood glucose analysis in *in vivo* study using experimental mice showed the anti-diabetic effect of TSCE by reducing the abnormal blood glucose level which was comparable to the standard anti-diabetic drug Glibenclamide. From this current work it is concluded that the tamarind seed coat extract (TSCE) could be used as an effective anti-diabetic drug. From this current study it is evident that the tamarind seed coat extract (TSCE) could be used as a novel anti-diabetic drug based on its *in vitro* and pre-clinical studies and further preclinical and clinical studies are required to strengthen their absolute Anti-diabetic activity.

AUTHORS’ CONTRIBUTIONS
Lakshminarayanan C. P. has carried out the entire study and drafting the manuscript. Prabhu S contributed in design of study and revising the manuscript.

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Declaration of interest
The authors report no declaration of interest.

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ABBREVIATIONS
TSCE – Tamarind Seed Coat Extracts
DPPH – 2, 2-diphenyl-1-picrylhydrazyl
FRAP – Ferric Reducing Antioxidant Potential
PTPB – Protein Tyrosine Phosphatase 1B
ACE – Angiotensin Converting Enzyme
DMSO – Dimethyl Sulfoxide
DPP-IV – Dipeptidyl Peptidases 4
SGLT2 – Sodium Glucose co-transporter
GLP1 – Glucagon like Protein 1
SU – Sulfonylureas
TZD – Thiazolidinediones
PNPP – Para Nitro Phenol Phosphate
ACE – Angiotensin Converting Enzyme