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PHARMACOGNOSTICAL, PHYTOCHEMICAL, *IN-VITRO* ANTIOXIDANT AND *IN-VIVO* ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACT OF STEM BARKS OF *CINNAMOMUM TAMALA*

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ABSTRACT

Traditional plant-based medicines are still needed by the whole world for their primary healthcare benefits. *Cinnamomum tamala* T. Nees & Eberm (Family Lauraceae) is commonly called as Indian bay leaf or Tejpatta. The leaves and bark of *Cinnamomum tamala* are used to cure various diseases due to its various properties including astringent, stimulant anthelmintic, diuretic, carminative and used in colic, dyspepsia, and diarrhea. Diabetes mellitus (DM) is a global health problem and the incidence of DM is increasing at alarming rate all over the world. Many Indian medicinal plants have been reported to possess potential antidiabetic activity and could play important role in the management diabetes. The objective of this study was to investigate pharmacognostical, phytochemical features, *in vitro* antioxidant activities and *in vivo* anti-diabetic potentials of methanolic extract of stem barks (*Cinnamomum tamala*) against streptozotocin-induced rat model collected from Bhopal region of Madhya Pradesh. The different pharmacognostical parameters were evaluated as per standard protocols with some modifications. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. The *in vitro* antioxidant activity of methanolic extract of the stem bark was assessed against DPPH and reducing power assay method using standard protocols. Rats were given streptozotocin (50mg/kg; i.p.) to induce diabetes, and glibenclamide (5mg/kg body weight) was utilized as the usual medication. Body weight and blood sugar levels were assessed in this study. Phytochemical analysis revealed the presence of alkaloids, glycoside, proteins and amino acids, flavonoids, tannin and phenolic compounds, saponins and carbohydrates. The activities of stem bark extracts against DPPH and reducing power assay method were concentration dependent. Oral treatment of methanolic extract of *Cinnamomum tamala* using rat oral needle at 200 and 400mg/kg doses significantly decreased blood glucose levels in diabetic rats than control rats and increase body wt. Hence, the chemical constituents of the plant extract might help in preventing diabetic complications and may serve as an alternative in the present armamentarium of antidiabetic drugs. Further study to substantiate the use of the plant as antidiabetic is recommended.

Keywords: *Cinnamomum tamala*, Lauraceae, Diabetes mellitus, Phytochemical analysis, Streptozotocin, Glibenclamide.

INTRODUCTION

Natural products obtained from the plant resources have been the major supplements to combat many serious diseases in the developing countries (Shihabudeen *et al.*, 2010). Free radicals and reactive oxygen species are well known inducers of molecular, cellular and tissue pathogenesis leading to several threats to the human society such as atherosclerosis, arthritis, cardiovascular diseases, central nervous system injury, gastritis, cancer, aging and Acquired Immune Deficiency Syndrome AIDS (Cook, 1996; Kumpulainen JT, Salonen, 1999; Tanaka *et al.*, 1998; Young, 2001). Along with lack of effective therapies, oxidative damage plays a decisive etiological factor in many chronic conditions, the expediency of antioxidants in protection against these diseases is defensible (Halliwell, 1994; Halliwell, 2007). Antioxidants are compounds capable either to delay or to inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are involved in the defence mechanism of the organism against the pathologies associated to the attacks of free radicals. Enzymes, like superoxide dismutase, catalase, glutathione peroxidase or non enzymatic compounds, such as uric acid, bilirubin, albumin etc have endogenous antioxidative potential. These endogenous antioxidative compounds lose its potential for controlling and providing the complete protection of the organisms against the reactive oxygen species, the need for exogenous antioxidants like natural antioxidants arises as a nutritional supplements or pharmaceutical products for their role in preventing human diseases (Singh and Naithani, 2014; Litescu *et al.,* 2011). The fruit juices, beverages and hot drinks obtained from the natural sources were found to reduce the morbidity and mortality caused by degenerative disorders as they are rich in antioxidants, like polyphenols, vitamin C, vitamin E, β-carotene and lycopene (Ramadan-Hassanien, 2008). There is an increasing interest in the measurement and use of plant antioxidants for scientific research as well as industrial (dietary, pharmaceutical and cosmetic) purposes in present time. This is basically due to strong biological activity, exceeding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis (Suhaj, 2006). Diabetes mellitus is the primary basis of, blindness, renal failure, strokes, lower limb and heart attacks amputations in adults around the world. In most developed countries, it ranks as the fourth leading cause of mortality. According to the International Diabetes Federation, 330 million people worldwide are expected to have diabetes by the year 2025, with Africa and Asia potentially experiencing the biggest increases. Developing nations will see this numerical growth. By 2025, more than 75% of diabetes would reside in developing countries, up from 62% in 1995 (Eseyin *et al.*, 2010). Diabetes is treated using insulin as well as a number of oral antidiabetic drugs such glinides biguanides, and sulfonylureas. One of the key areas of research is the hunt for more powerful and secure hypoglycemic agents because many of them have a lot of major side effects (Patel *et al.*, 2012). Orally consumed herbs and plants have been said to

have hypoglycemic properties (Rajan *et al.*, 2012). According to the WHO, more than 1200 plant species are used to treat diabetes mellitus worldwide, and a sizable number of these plants shown beneficial hypoglycemic action during laboratory testing (Rajasekar *et al.*, 2010), certain medicinal plants have recently been shown to be effective in treating diabetes worldwide and have been empirically used in anti-hyperlipidemic and anti-diabetic therapy. Finding new anti-diabetic drugs made from natural plants is still intriguing even if there are more than 400 plant species with hypoglycemic activity described in literature. This is because these plants include chemicals that have various and safe effects on diabetes mellitus. Alkaloids, terpenoids, glycosides, carotenoids, and flavonoids, among other compounds, are present in the majority of plants and well acknowledged to have antidiabetic properties (Malviya *et al.*, 2010). However, research into plants hypoglycemic, antioxidant, and hypolipidemic, qualities may lead to the creation of fresh pharmaceutical treatments for the treatment of diabetes mellitus (Dangi and Mishra, 2010). Streptozotocin (STZ) one of the most widely used diabetogenic drugs is (Yalniz and Pour, 2006), an antibiotic produced by *Streptomycetes achromogenes* that is inexpensive and has few side effects (Ghasemi *et al.*, 2014). When coupled with nicotinamide (NA), it is used to cause both type 1 and type 2 diabetes (Nayak *et al.*, 2014). Pancreatic cells are highly selectively poisonous to it. The hexose moiety, which aids it in crossing the cell membrane via GLUT2 (Yalniz and Pour, 2006), is responsible for selectivity. DNA is alkylated by STZ by the transfer of the CH_3 group to the DNA particle, which fragments the DNA and contributes to its toxicity. This excessively activates poly (ADP-ribose) polymerase-1 (PARP-1), which reduces cellular NAD+ (the substrate of PARP), ATP, and ultimately leads to beta cell necrosis. Additional mechanisms by which STZ is damaging include the generation of reactive oxygen species (ROS), nitric oxide, NF-B, the reduction of mitochondrial membrane potential, and the activation of c-Jun N-terminal kinase (Eleazu *et al.*, 2013). *Cinnamomum tamala* belonging to family Lauraceae is a widely used medicinal plant to treat various diseases. Its local name is Tezpat (Urdu). The leaves of this plant have also been extensively used extensively as spice in the foods industry due to its special aroma (Chang and Cheng, 2002). *Cinnamomum tamala* has been studied for its phytochemical constituents by different researchers. Prakash *et al.*, (2014) presented the result of the phytochemical analysis of the hexane, methanol, acetone and chloroform extracts of the *Cinnamomum tamala* leaves extracts. They detected alkaloid and tannins but didn't spot glycosides in there samples. Flavonoid were detected from methanol, acetone and chloroform extracts but were absent in hexane extracts. Sterol were detected from hexane, methanol and acetone but was not detected in chloroform extracts. Similarly, Sukumar and his coworkers reported polyphenoles, flavonoids, tannins, alkaloids and saponins (Dandapat *et al.*, 2014). In Ayurvedic medicine *Cinnamomum tamala* were used in the treatment of diarrhea, anorexia, coryza and dryness of mouth. *Cinnamomum tamala*

leaves are antihelminthic, diuretic and are good for spleen and lever as well as useful in Inflammation. The medicinal uses also include as an antiflatulent, carminative and in the treatment of cardiac disorders (Singh *et al.,* 2012). Besides these, various pharmacological activities have been reported in natural products from *cinnamomum* species. The essential oil from *Cinnamomum tamala* exhibits antidermatophytic (Yadav *et al.,* 1999), antibacterial (Goyal *et al.,* 2009), antifungal, antihyperglycaemic and (antihypercholesterolanemic) effect (Srivastava *et al.,* 2011). The aim of this study was to assess the quality (types), *in vitro* antioxidant activities and antidiabetic potential of the methanolic extract of *Cinnamomum tamala* stem bark in diabetic rats.

MATERIALS & METHODS

Chemical reagents

All the chemicals used in this investigation were given by Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India), Hi Media Laboratories Pvt. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India). Glibenclamide (Unichem, Ltd. (Alkem, Mumbai); streptozotocin; only analytical-grade substances were used in the investigation.

Pharmacognostical evaluation (Evans, 1997)

Total ash value

About 5 gm each of powdered parts were accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air dried powder.

$$\% \text{ Ash content} = \frac{\text{Weight of crucible + ash} - \text{Weight of crucible}}{\text{Weight of crucible + sample} - \text{Weight of crucible}} \times 100$$

Acid insoluble ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

$$\% \text{ Acid soluble ash} = \frac{\text{Weight of crucible + ash} - \text{Weight of crucible}}{\text{Crude drug weight}} \times 100$$

Water insoluble ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated

with reference to air-dried parts respectively (Evans, 1997).

% Water soluble ash =Weight of crucible + ash – Weight of crucible × 100
Crude drug weight

Loss on drying

Place 2 to 6 g of the sample into a weighing bottle which has been accurately weighed, and weigh it accurately. Then, dry it at 105 o C for 5 - 6 hours and cool it in desiccators with silica gel. When the material is dried to a constant weight, the percent of loss on drying is determined (Evans, 1997).

LOD % = Wt. of petridish + crude drug - After drying Wt. of petridish + sample × 100
Weight of crude drug

Alcoholic extractive value

5g of powdered material was weighed into 250mL stopper conical flask containing 100mL of 90% ethanol and the stopper replaced. The flask and content was placed in a mechanical shaker for 6hrs and then allowed to stand for 18hrs. The mixture was filtered and 20mL of the filtrate was measured into an evaporating dish with a known weight, and evaporated to dryness. The constant weight of the residue was gotten after drying in the oven at 1050C for about 3 minutes. The extractive value was calculated.

Water soluble extractive value = Weight of reside /Weight of the drug ×100

Water extractive value

The procedure was the same as above except that water used in place of 90% ethanol.

Water soluble extractive value = Weight of residue /Weight of the drug × 100

Extraction

Plant material fattening

Plant matter from *Cinnamomum tamala* was crushed up and allowed to air dry at ambient temperature. Soxhlation was used to remove the substance from the shade-dried plants using petroleum ether after it had been coarsely crushed up. The substance was extracted repeatedly until it had been adequately fatted.

Extraction by soxhlation process

Cinnamomum tamala powder that has been defatted was thoroughly extracted with methanol using the soxhlation process. The extract evaporated beyond their boiling points. The dried crude concentrated extract was weighed in order to calculate the extractive yield. When ready for analysis, it was then put into glass vials (6 x 2 cm) and stored in a refrigerator 4°C (Jain *et al.*, 2010)

Phytochemical screening

According to the protocols described, phytochemical screening was done to find any bioactive compounds (Dutta *et al.*, 2020; Gadekar *et al.*, 2020). By visually seeing a colour change or the production of precipitates following the addition of specific reagents to the solution, the tests were recognized.

Antioxidant activity

DPPH radical scavenging activity

0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared. Freshly 1 mg/ml methanol solution of all extracts of *Cinnamomum tamala* /standard was prepared. 1 mg of extracts/standard was taken with methanol to make 1mg/ml stock solution. Different volume of extracts/standard (20-100µl) was taken from stock solution in a set of test tubes

and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature. For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm (Gulcin *et al.*, 2006). Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

Reducing power assay

A spectrophotometric method was used for the measurement of reducing power. For this 0.5 ml of each of the extracts was mixed with 0.5ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of 1% potassium ferricyanide (10 mg/ml). The reaction mixture was incubated at 50ºC for 20 min separately and then rapidly cooled, mixed with 1.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (0.5ml) of the supernatant was diluted with distilled water (0.5ml) and then ferric chloride (0.5ml, 0.1%) was added and allowed to stand for 10 min. the absorbance was read spectrophotometrically at 700 nm. Ascorbic acid (AA) was used as standard for construction of calibration curve (Jain and Jain, 2011).

Reducing Power (%) = (As / Ac) × 100

Here, Ac is the absorbance of control (AA) and as is the absorbance of samples (extracts) or standards.

Animals

Wistar rats (200±50 gm) were residence in groups of six under regulated humidity and temperature settings (25±2 °C, 55-65%). Rats were given regular rodent food and unlimited amounts of water. Prior to the experiments, rats spent 7 days becoming used to the lab environment. Between 8:00 and 15:00 hours, all studies were conducted in a room with no background noise. Each set of studies used a different group of rats (n=6). The Institutional Animal Ethics Committee (IAEC), established by the India's Ministry of Environment and Forests, located in New Delhi to oversee and supervise the use of experimental animals, gave its approval to the animal experiments.

Streptozotocin (STZ) induced diabetes

STZ solution of 10mg/ml was prepared in ice-cold citrate buffer 0.1M, Ph 4.5 and given intraperitoneally at a dose of 50mg/kg body weight within 5 minutes. Rats with moderate diabetes, glycosuriya, and hyperglycaemia (i.e. blood glucose of 200-300 mg/dl) were used in the experiment 48 hours after receiving STZ. Blood samples were drawn from retro-orbital mode of blood collection of rat in the study. Fasting blood glucose estimation will be performed on day 0, 3, 7, 14 and 21 of the study. For the estimation of blood glucose level Accu-Check Active glucometer (A product of Roche Diagnostics, Germany) will be used, where the blood glucose level are expressed in mg/dl. This method has adequate sensitivity with the advantage that a small amount of blood (1-2 µL) can be used for blood glucose analysis. Blood sample will be collected by retro-orbital and put on the glucose test strip on the glucometer (Etuk, 2010).

The animals were randomly divided into following five groups; each group consists of six animals. Animal grouping and their treatment is as follows:

Group-I: Normal Control: - treated with normal saline.

Group-II: Negative Control:- Streptozotocin-induced diabetic rats in which streptozotocin (50 mg/kg was administrated i.e. in 0.1M sodium citrate) treated with normal saline.

Group-III: Test-1: STZ-induced diabetic rats treated with 200 mg/kg *Cinnamomum tamala*

Group-IV: Test-2:-STZ-induced diabetic rats treated with 400mg/kg *Cinnamomum tamala*

Group-V: Standard: -STZ-induced diabetic rats treated with Glibenclamide (5mg / kg) orally

Diabetes was induced by a single i.p. injection of streptozotocin a dose of 50 mg/kg body weight. Except Group I all the other 4 groups were induced with diabetes. Glibenclamide were suspended in 0.9% NaCl in warm water as vehicle solution and administered orally for 21days. The treatment schedule was begun on 4th day after diabetic induction and it was counted as 1st day of treatment. It was continued till 21 days. Body weight and level of glucose in blood were observed on 0, 3, 7, 14 and 21 days of post treatment. After overnight fasting, diabetes was induced by intraperitoneal injection of STZ dissolved in 0.1M cold sodium citrate buffer, pH 4.5, at a dose of 50 mg/kg and the doses were determined according to the body weight of animals. In the present study the blood glucose levels were evaluated in all the rats prior to administration of STZ. On day 3 i.e. after 72 hours, the blood glucose levels were evaluated and the rats with blood glucose level >250 mg/dl were considered as diabetic and taken up for the study (Kumar *et al.*, 2012).

Collection of blood sample and blood glucose determination

Blood samples were drawn from tail tip of rat in the study. Fasting blood glucose estimation was done on day 0, 3, 7, 14 and 21 of the study. For the estimation of blood glucose level,Accu-Check Active glucometer (A product of Roche Diagnostics, Germany) was used, where the blood glucose level were expressed in mg/dl. This method has adequate sensitivity with the advantage that a small amount of blood (1-2 μL) can be used for blood glucose analysis. Blood sample was collected by cutting the tail tips with a sharp blade and put on the glucose test strip on the glucometer.

Statistical analysis

The mean and SEM were employed to state all the data. One-way analysis of variance was employed to evaluate the groups' statistical implication before Dunnett's t-test post-hoc analysis. P values below 0.5 were regarded as noteworthy.

RESULTS AND DISCUSSION

After completing each consecutive soxhlation extraction process, the crude extracts were focused on a bath of water by totally evaporating the solvents to achieve the real extraction capitulate. Petroleum ether and methanol were found to produce extracts from plant portions called leaves with yields of 0.475 and 5.843%, respectively. *Cinnamomum tamala* stem bark were shade dried and ground into powder to measure a number of physiochemical parameters, including loss on drying, total ash value,

extractive soluble in alcohol, extractive soluble in water, and foaming index (Table 1). Table 2 shows the results of a qualitative phytochemical analysis of the raw stem bark of *Cinnamomum tamala*. Alkaloids, glycoside, proteins and amino acids, flavonoids, tannin and phenolic compounds, saponins and carbohydrates were all detected in methanolic plant extracts. Antioxidant activity of the samples was calculated through DPPH assay and reducing power assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard in both the tests and the values were comparable with concentration ranging from 20µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed. % inhibition was higher in the ascorbic acid while the values were lesser in methanolic extract Table 3. The reducing ability of the compound usually depends on the reductants, which have been exhibited antioxidative capacity by breaking the free radical chain, donating a hydrogen atom. Reducing power assay was calculated in extracts and the values indicated a superior activity Table 4. The effects of the extract on body weight in diabetic rat are shown in Table 5. All groups prior to extract administration showed no apparent difference in body weight compared to normal control group. Significant body weight gain was recorded for *Cinnamomum tamala* 200 and 400 mg/kg at the 9th day of treatment compared to diabetic control group. All doses of the extract and standard showed a significant improvement in body weight at the 21th day when compared to diabetic control. By contrast, the body weight of the diabetic control group was significantly decreased at the 21th day compared to a normal control group. On day 9^{th} glucose levels were significantly decreased for glibenclamide treated group (150.39) when compared with control group at 7th and 14^{th} days. The blood glucose level of methanolic extract of *Cinnamomum tamala* treated groups 200 mg/kg and 400 mg/kg were 238.25 and 216.14 when compared with control group at 9^{th}day. The methanolic extract of *Cinnamomum tamala at* 200 mg/kg and 400 mg/kg have been expressed dose dependent anti diabetic action when compared to normal control and standard Glibenclamide treated group. On 14^{th} day, the blood glucose level of methanolic extract of *C. tamala* (200 mg/kg and 400 mg/kg) was found to be 201.14 and 166.14 mg/dl. On day 21^{st}, methanolic extract of *Cinnamomum tamala* treated animals at dose level of 200 & 400 mg/kg significantly decreased and maintain the blood glucose level (180.45 and 144.85) which is comparable to positive control Table 6.

Table 1: Pharmacognostic evaluation of stem bark extract of *Cinnamomum tamala*

Parameters	Value in percentage (%)
Ash Value	10.9
Water insoluble ash	2.53
Acid insoluble ash	2.45
Loss on drying	8.59
Alcoholic extractive value	3.49
Water extractive value	13.85
Foaming index	24 (ml)

Table 2: Phytochemical testing of *Cinnamomum tamala* extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	**Methanolic extract**
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Absent	Present
2.2	Legal's test	Absent	Present
2.3	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Absent	Present
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponins**		
7.1	Foam test	Absent	Present
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Absent	Absent
8.2	Libbermann-Burchard's test	Absent	Absent

Table 3: DPPH assay of ascorbic acid and methanolic extract

S. No.	Conc. (µg/ml)	Ascorbic acid (% Inhibition)	Methanolic Extract (% Inhibition)
1.	20	50.26853	41.768
2.	40	56.6058	46.2983
3.	60	66.058	54.1436
4.	80	71.96563	57.0166
5.	100	85.49946	65.1934
IC 50 Value		**22.54**	**50.13**

Table 4: Result of reducing power assay

S. No.	Conc. (µg/ml)	Ascorbic acid	Methanolic Extract
1.	**20**	0.14	0.025
2.	**40**	0.244	0.085
3.	**60**	0.367	0.09
4.	**80**	0.43	0.12
5.	**100**	0.505	0.145

Table 5: Effect of *Cinnamomum tamala* extract on Body weight of the rats

Treatment groups	Body Weight (gms)				
	0 day	**3 day**	**9 day**	**14 day**	**21 day**
Normal Control	212.45±0.15	214.64±0.48	215.45±0.35	218.31±0.25	220.49±0.85
STZ treated	181.26±1.25	171.19±0.16	172.16±0.35	169.16±0.14	160.14±1.25
Glibenclamide treated	161.85±0.14	190.51±1.47	218.48±1.85	217.14±1.47	214.28±1.96
C.tamala treated (200 mg/kg bw)	163.15±1.85	168.25±1.87	172.26±1.69	175.41±1.35	182.15±1.58
C. tamala treated (400 mg/kg bw)	160.29±2.14	165.16±2.36	176.28±2.65	182.28±2.36	190.14±1.62

Table 6: Effect of test samples of extract on blood glucose level in experimental rats

Treatment groups	Blood Glucose Level				
	0 day	3 day	9 day	14 day	21 day
Normal Control	121.12±1.25	115.25±2.15	131.36±0.15	108.45±1.58	118.14±1.25
STZ treated	120.45±0.25	150.14±1.45	216.14±1.58	288.14±2.45	286.14±2.85
Glibenclamide treated	288.45±1.45	210.25±1.79	150.39±2.46	130.48±2.69	116.12±2.43
C. tamala treated (200 mg/kg bw)	276.14±0.15	254.14±3.47	238.25±3.49	201.14±3.52	180.45±3.17
C. tamala treated (400 mg/kg bw)	284.48±0.14	243.43±1.58	216.14±2.76	166.14±0.47	144.85±3.48

CONCLUSION

The present investigation discusses the diabetic potential of the *Cinnamomum tamala* in STZ - induced diabetic rat. The use of STZ to induce diabetes mellitus in rodent models is widely accepted and STZ induced diabetes is reported to resemble human diabetes mellitus which is characterized by glycosuria, hyperglycemia, polyphagia, polydipsia, and body weight loss when compared with normal rodents. Streptozotocin is by far the most common and well established chemical model used for induction of experimental diabetes. It is a better diabetogenic agent than alloxan as it is linked with wider species effectiveness and greater reproducibility. The diabetogenic action of STZ is mainly, due to the DNA alkylating activity of its methylnitrosourea moiety, release of nitric oxide from the nitroso group in its further course of action and generation of reactive oxygen species. In the present study, STZ at a dose of 50 mg/kg body weight was used to induce experimental diabetes and it led to an elevated level of plasma BGL. Glibenclamide is often used as a standard antidiabetic drug in STZ -induced moderate diabetes to compare the antidiabetic effects of a variety of bioactive compounds. Similarly, oral administration of *Cinnamomum tamala* stem bark extract at all tested doses (200 and 400 mg/kg) and glibenclamide at a dose of 5 mg/kg produced a significant reduction in blood glucose level of diabetic mice. The reduction in BGL was dose and time dependent with the maximum reduction in BGL (144.85±3.48) achieved at the highest dose (400 mg/kg), which was comparable to glibenclamide (116.12±2.43) at a dose of 5 mg/kg. This result indicates that the crude extract can exhibit hypoglycemic activity in normal mice which is in agreement with other studies performed on similar species. It has been reported that methanolic stem bark extracts of *Cinnamomum tamala* had a significant hypoglycemic activity in diabetic rats. The plant might possess an insulin-like effect or stimulate insulin secretion from β -cells. Compounds of a

natural product such as flavonoid and tannins isolated from medicinal plants are reported to stimulate insulin secretion from pancreatic β - cells. Since these active constituents exist in *Cinnamomum tamala* the probable mechanism of action of plant is similar to glibenclamide. STZ-induced DM is characterized by loss of body weight possibly due to the inability of cells to utilize glucose, lipolysis in adipose tissue and protein break down which leads to skeletal muscle wasting. Likewise, in the present study, STZ caused a massive percentage of weight loss in the diabetic control groups. Such weight loss was ameliorated by the crude extract at all dose levels (200, 400 mg/kg) as compared to the diabetic control group even though the effect was not statistically significant. The protective effect of the *Cinnamomum tamala* crude extract and the solvent fractions on body weight loss could be explained due to their ability to reduce hyperglycemia. Natural products play a major role in the discovery of new therapeutic agents and have received much attention as sources of bioactive substances including antioxidants, hypoglycemic, and hypolipidemic agents. Preliminary phytochemical screening of *Cinnamomum tamala* extract showed the presence of alkaloids, Flavonoid terpenoids, tannins, phenolic compounds and saponins. The significant antidiabetic and antioxidant activity of *Cinnamomum tamala* in this study may be attributed to the presence of these principal constituents. Increased free radical generation and oxidative stress are hypothesized to play an important role in the pathogenesis of diabetes and it's late to play an important role in the pathogenesis of diabetes and its late complications. Extract of stem bark of *Cinnamomum tamala* exhibit significant antidiabetic effects in STZ-induced rats. The *Cinnamomum tamala* extracts with each dose of 200 and 400 mg/kg body weight had antidiabetic effects on STZ-induced diabetes on rats. On day 21st, methanolic extract of *Cinnamomum tamala* treated animals at dose level of 200 & 400 mg/kg significantly decreased and maintain the blood glucose level (180.45±3.17 and 144.85±3.48) which is comparable to positive control. Hence, the chemical constituents of the plant extract might help in preventing diabetic complications and may serve as an alternative in the present armamentarium of antidiabetic drugs. Further chemical and pharmacological investigations are required to elucidate the exact mechanism of action of this extract and to isolate the active principles responsible for such effects. At present, work is in process to isolate the active constituents responsible for such effects.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Shihabudeen, M.S., Priscilla, H.H.D. & Thirumurugan, K. (2010) Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. *International Journal of Pharmaceutical Sciences and Research*, 1, 430–434.

- Cook, N. (1996) Flavonoids—Chemistry, metabolism, cardioprotective effects, and dietary sources. *Journal of Nutritional Biochemistry*, 7, 66–76
- Kumpulainen, J.T. & Salonen, J.T. *Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease 1999*.
- Tanaka, T., Kusano, R. & Kouno, I. (1998) Synthesis and antioxidant activity of novel amphipathic derivatives of tea polyphenol. *Bioorganic and Medicinal Chemistry Letters*, 8, 1801–1806
- Young, I.S. & Woodside, J.V. (2001) Antioxidants in health and disease. *Journal of Clinical Pathology*, 54, 176–186
- Halliwell, B. (1994) Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? *Lancet*, 344, 721–724
- Halliwell, B. (2007) Biochemistry of oxidative stress. *Biochemical Society Transactions*, 35, 1147–1150
- Singh, M. & Naithani, M. (2014) Phytochemical estimation and antioxidant activity of seed extract of millets traditionally consumed by common people of Uttrakhand, India. Int. *J. Biol* .Pharm .Allied Sci., 3, 2389–2400.
- Litescu, S.C., Eremia, S.A.V., Sav, D.M., Tache, A. & Radu, G.L. (2011) Biosensors applications on assessment of reactive oxygen species and antioxidants. In: (edited by V. Somerset). Environmental Biosensors.In tech open, pp. 95–114.
- Ramadan-Hassanien, M.F. (2008) Total antioxidant potential of juices, beverages and hot drinks consumed in Egypt screened by DPPH in vitro assay. *Grasas y Aceites*, 59, 254–259
- Suhaj, M. (2006) Spice antioxidants isolation and their antiradical activity: A review. *Journal of Food Composition and Analysis*, 19, 531–537
- Eseyin, O., Ebong, P., Eyong, E., Awofisayo, O. & Agboke, A. (2010) Effect of Telfairia occidentalis on oral glucose tolerance in rats. *African Journal of Pharmacy and Pharmacology*, 4, 368–372.
- Patel, D.K., Prasad, S.K., Kumar, R. & Hemalatha, S. (2012) An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pacific Journal of Tropical Biomedicine*, 2, 320–330
- Rajan, M., Kumar, V.K., Kumar, P.S., Swathi, K.R. & Haritha, S. (2012) Antidiabetic, antihyperlipidaemic and hepatoprotective activity of methanolic extract of Ruellia tuberose Linn. leaves in normal and alloxan induced diabetes. *Journal of Chemical and Pharmaceutical Research*, 4, 2860–2868.
- Rajasekar, V., Kirbanandan, S. & Lakshmi, B.S. (2010) Assessment of antidiabetic activity of Syzygium jambolanum using in vitro model. *American Journal of Infectious Diseases*, 5, 99–105.

- Malviya, N., Jain, S. & Malviya, S. (2010) Antidiabetic potential of medicinal plants. *Acta Poloniae Pharmaceutica*, 67, 113–118
- Dangi, K.S. & Mishra, S.N. (2010) Antihyperglycemic, antioxidant and hypolipidemic effect of Capparis aphylla stem extracts in streptozotocin induced diabetic rats. *Biology and Medicine*, 2, 35–44.
- Yalniz, M. & Pour, P.M. (2006). *Toxicology of the Endocrine Pancreas*. Taylor & Francis Group: Boca Raton, FL, USA, pp. 542–545.
- Ghasemi, A., Khalifi, S. & Jedi, S. (2014) Streptozotocin-nicotinamide-induced rat model of type 2 diabetes [Review]. *Acta Physiologica Hungarica*, 101, 408–420
- Nayak, Y., Hillemane, V., Daroji, V.K., Jayashree, B.S. & Unnikrishnan, M.K. (2014) Antidiabetic activity of benzopyrone analogues in nicotinamide-streptozotocin induced type 2 diabetes in rats. *TheScientificWorldJournal*, 2014, 854267
- Eleazu, C.O., Eleazu, K.C., Chukwuma, S. & Essien, U.N. (2013) Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. *Journal of Diabetes and Metabolic Disorders*, 12, 60
- Chang, S.T. & Cheng, S.S. (2002) Antitermitic activity of leaf essential oils and components from Cinnamomum osmophleum. *Journal of Agricultural and Food Chemistry*, 50, 1389–1392
- Prakash (2014) Phytochemical analysis of Cinnamomum tamala of leaves extract. *Indian Streams Research Journal*, 4, 1–3.
- Dandapat, S., Kumar, M. & Sinha, M.P. (2014) HerapeutLc efficacy of Cinnamomum tamala (Buch.-Ham.) and Aegle marmelos (L.) leaf. *Balneo Research Journal*, 5, 113–122
- Singh, V., Gupta, A.K., Singh, S.P. & Kumar, A. (2012) Direct analysis in real time by mass spectrometric technique for determining the variation in metabolite profiles of Cinnamomum tamalaNees and Eberm genotypes. *TheScientificWorldJournal*, 2012, 549265
- Yadav, P., Dubey, N.K., Joshi, V.K., Chansouria, J.P.N. & Yadav, P. (1999) Antidermatophytic activity of essential oil of Cinnamomum. *Journal of Medicinal and Aromatic Plant Sciences*, 21, 347–351.
- Goyal, P., Chauhan, A. & Kaushik, P. (2009) Laboratory evaluation of crude extracts of Cinnamomum tamala for potential antibacterial activity. *Electronic Journal of Biology*, 5, 75–79.
- Srivastava, B., Sagar, A. & Dubey, N.K. (2011) Evaluation of Cinnamomum tamalaoil and its phenylpropanoideugenol for their antifungal and antLaflatoxLgenLc activity. *Food Analytical Methods*, 4, 347–356

- Evans, W.C. (1997) Trease and Evans' pharmacognosy. *General Pharmacology*, 2, 291.
- Jain, D.K., Gupta, S., Jain, R. & Jain, N. (2010) Anti-inflammatory activity of 80% ethanolic extract of Acorus calamus Linn. Leaves in albino rats. *Research Journal of Pharmacy and Technology*, 3, 882–884.
- Dutta, R., Sharma, M.K., Khan, A. & Jha, M. (2020) Phytochemical and in vitro antioxidant assay of Fumaria officinalis leaf extract. *Journal of Advanced Scientific Research*, 11, 176–182.
- Gadekar, S., Goyal, S., Khan, A. & Jha, M. (2020) Chemopreventive action of Sphaeranthus indicus on dmba-induced skin papillomagenesis in mice. *Journal of Advanced Scientific Research*, 11, 161–167.
- Gulçin, İ., Elias, R., Gepdiremen, A. & Boyer, L. (2006) Antioxidant activity of lignans from fringe tree (Chionanthus virginicus L.). *European Food Research and Technology*, 223, 759–767
- Jain, R. & Jain, S.K. (2011) Total phenolic contents and antioxidant activities of some selected anticancer medicinal plants from Chhattisgarh State, India. *Pharmacologyonline*, 2, 755–762.
- Etuk, E.U. (2010) Animals models for studying diabetes mellitus. *Agriculture and Biology Journal of North America*, 1, 130–134.
- Kumar, S., Kumar, V. & Prakash, O.M. (2012) Antidiabetic and hypolipidemic activities of Kigelia pinnata flowers extract in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine*, 2, 543–546