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# EVALUATION OF ANTI-OXIDANT AND ANTI-DIABETIC ACTIVITIES OF *CINNAMOMUM TAMALA* LINN LEAVES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

Palanisamy P[1]*, K.R. Srinath[2], D. Yoganand Kumar[3], Pooja Chowdary C[4]
[1]Vinayaka Mission's College of Pharmacy, Vinayaka Missions University, Salem, Tamil Nadu, India
[2]Campbell University, North Carolina, USA-27506
[3]Trainee Research Associate - Pharmacokinetics/Biostatistics, Axis Clinicals Ltd, Hyderabad, Andhra Pradesh, India
[4]Pulla Reddy Institute of Pharmacy, Dundigal, Medak, AP, India

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*Email: palanisamy2907gmail.com

**ABSTRACT**
The main objective of this study was to focus on the anti-diabetic activity of *Cinnamomum tamala* Linn, with special reference to its curative and protective role in streptozotocin- induced diabetic animal model. Attempts were further made to study the antioxidant properties *of Cinnamomum tamala* Linn. leaves. The efficacy of 50% ethanolic extract of leaves of *Cinnamomum tamala* showed significant decrease in the blood glucose level and increase in the antioxidant efficacy in streptozotocin induced diabetes. It was demonstrated that the oral administration of the 50% ethanolic extract of leaves of *Cinnamomum tamala* extract to streptozotocin induced diabetic rats showed significant positive changes in the biochemical and physiological parameters related to carbohydrate, protein and lipid metabolism., the strong antihyperglycemic and the anti-oxidant effect observed in streptozotocin-induced diabetic rats justified the use of 50% ethanolic extract of leaves of *Cinnamomum tamala* for the treatment of diabetes-related complications.

**Keywords**: antidiabetic, *Cinnamom tamala* Linn., anti-oxidant.

## INTRODUCTION

Diabetes Mellitus (DM), is the most common endocrine chronic disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced.. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves. The aim is to develop a scientifically validated and standardized herbal ration which will be useful in the treatment of diabetes. The main objective of this study was to focus on the anti-diabetic activity of *Cinnamom tamala* Linn, with special reference to its curative and protective role in streptozotocin- induced diabetic animal model. Attempts were then further made to study antioxidant properties *of Cinnamom tamala* Linn. leaves.

## MATERIALS AND METHODS

### Plant material

The leaves of *Cinnamoamum tamala* (Family -Liliaceae) were collected from Garden of National Botanical Research Institute, Lucknow, India and was authenticated by Dr. Sayeeda Khatoon, chemotaxonomist .

### Animals

Sprague-Dawley rats (150-185g) and Swiss albino mice (20-25 gm) of either sex and of approximately the same age were procured from the animal house of Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at 26 ± 2 °C and relative humidity 44 – 56 % in polypropylene cages. The animals were exposed to alternate 12 hrs of darkness and light each..Animals were provided with standard rodent pellet diet (Dayal, India) and the food was withdrawn 18-24 h before the experiment though water was allowed *ad libitum*. All experiments were performed in the morning according to current guidelines for investigation of experimental pain in conscious animals. The standard orogastric cannula was used for oral drug administration in experimental animals[1].

### Preparation of plant extract

Method of extraction: Cold percolation process.
Requirements: Percolater, Shade dried coarse powder of leaves of *Cinnamoamum tamala* .
Solvents: Petroleum ether and 50% ethanol.

### Preliminary Phytochemical Screening

50% ethanolic extract of *Cinnamoamum tamala*. was subjected to qualitative tests for the identification of various active constituents viz. carbohydrate, glycoside, alkaloid, amino acids, flavanoids, fixed oil, tannins, gum and mucilage, phytosterols etc. according to Khandelwal[2].

### Acute toxicity studies (OECD Guideline 423)

This test involves the administration of a simple bolus dose of test substances to faster healthy young adult rodents by oral gavage, observation for upto 15days after dosing and recording of body weight and the necropsy of all the animals. In this method pre-specified fixed doses of the test substances were used ie, 5mg/Kg, 50mg/Kg, 300mg/Kg, 2000mg/Kg and the mortality due to these doses were observed. Generally female animals were used for this study and each dose group should consist of 3 animals.

### *In Vitro* Antidiabetic Activity[3]

### α – Amylase Inhibition Activity

Alpha amylase enzyme is responsible for the metabolism of polysaccharide such as starch carbohydrate etc. the aim behind present experiment is to study the effect of α-amylase concentration on the rate of reaction and inhibition activity of 50% ethanolic extract of *Cinnamoamum tamala* leaves.

### *In Vivo* Antidiabetic Activity

### Experimental induction of diabetes[4]

All animals were allowed to adapt to metabolic cages for 3 days, after which they were fasted overnight and 50 mg/kg of streptozotocine (Sigma, St. Louis, MO, USA) freshly dissolved in normal saline was injected intraperitoneally. After treatment, all animals were returned to their cages and given free access to food and water. Blood glucose levels were measured 3 days after streptozotocine injection and used as parameter to obtain matching pairs of rats with diabetes of similar level of severity. Only rats with fasting blood glucose levels greater than 200 mg/dL were considered diabetic and then included in this study. The mean blood concentration of glucose in normoglycemic rats was 95 mg/dL. Diabetic rats were randomly assigned to four different groups ($n$ = 6 animal/groups). All treatments started 3 days after streptozotocine injection.

Group I - Control rats received vehicle solution (1% carboxy methyl cellulose)

Group II - Diabetic control rats received vehicle solution (1% carboxy methyl cellulose)
Group III - Diabetic rats treated with extract 200 mg/kg body weight in 1% carboxy methyl cellulose
Group IV - Diabetic rats treated with extract 400 mg/kg body weight in 1% carboxy methyl cellulose
Group V - Diabetic rats treated with Glibenclamide 600 µg/kg body weight in aqueous solution

**Glucose estimation**

The collected serum samples of different study group were subjected to the serum glucose level estimation by enzymatic GOD-POD method by using glucose diagnostic kit (Auto-span).

**Total cholesterol estimation**

The serum cholesterol level was estimated by wybenga and pileggi method using cholesterol diagnostic reagent kit (span)

**Triglycerides estimation**

The triglycerides level was estimated by Glycerol phosphate oxidase (GPO) method.

**Bio-chemical estimation**

Serum was analyzed for the following parameters aspartate aminotransferase/serum glutamic oxaloacetic transaminase (ASAT)/(SGOT), alanine amino transferase/serum glutamate pyruvate transaminase (ALAT)/(SGPT), alkaline phosphatase (ALP) and cholesterol.

***In Vitro* Antioxidant Activity**[5]

**Free radical scavenging ability (FRSA) by the use of DPPH (1, 1-diphenyl-2 picrilhydrazyl)**

The DPPH radical scavenging activity of PLANT was determined by using the method proposed by Yen and Duh (1994). Aliquot of 20 - 80 µl (5 mg/ 10 ml) was added to 2.9 ml of freshly prepared solution of DPPH (6 x $10^{-5}$ M in MeOH). The Absorbance was recorded at $\lambda_{max}$ 517 nm after 1 hour of incubation.

***In Vivo* Antioxidant Activity**

**Assay of lipid peroxidation**

The concentration of thiobarbituric acid reactive substances (TBARS) was measured (lipid peroxidation product maondialdehyde (MDA) was estimated) in liver using the method of Okhawa et al[6]

**Catalase activity**

CAT activity was determined by monitoring the enzyme-catalyzed decomposition if hydrogen peroxide by potassium permanganate according to Cohen et al.[7]

**Superoxide dismutase activity**

SOD was estimated using the standard method of Kakkar et al.[8].

**Glutathione peroxidase**

The incubation mixture at 37 ° C contained 0.08 M sodium phosphate (pH 7.0), 0.08 M EDTA, 1.0 mM sodium azide, 0.4 nM GSH and 0.25 mM $H_2O_2$. GSH was determined at 3 minute intervals using DTNB. An enzyme unit represents a decrease in GSH concentration of 0.001 log unit per minute after subtraction of nonenzymic mode.

**Statistical Analysis**

All the values were expressed as mean ± SEM (standard error mean) for six rats. Statistical analysis was carried out by using PRISM software package (version 3.0). Statistical significance of differences between the control and experimental groups was assessed by One-way ANOVA followed by Newman-Keuls Multiple Comparision Test. The value of probability less than 5% ($P < 0.05$) was considered statically significant.

**Histopathological Studies**

Section of the pancreas and kidney tissues were made, stained with Haematoxylin and Eosin reagent and observed under low and high power objective for histopathological changes. The alteration and changes in the histology of pancreas and kidney were shown in vide plate

## RESULTS

The phytoconstituents were extracted by using different solvent of increasing polarity like Petroleum ether, Ethanolic(50%).The extractive values were presented in table no.1

**Table No 1. Percentage yield of various extracts of leaves of *Cinnamomum tamala***

| Plant used | Part used | Method | Percentage yield | |
|---|---|---|---|---|
| | | | Petroleum ether | 50% ethanol |
| *Cinnamomum tamala* | Leaves | Cold percolation | 3.35%w/w | 9.75% w/w |

**Table No. 2. Preliminary phytochemical screening**

| S.No | Constituents | Tests | Petroleum ether Extract | 50%Ethanolic extract |
|---|---|---|---|---|
| 1. | Carbohydrate | Molish's test | + | + |
| | | Fehling's test | + | + |
| 2. | Fixed oil & fats | Spot test | + | + |
| | | Saponification test | + | + |
| 4. | Proteins & amino acids | Million's test | _ | _ |
| | | Ninhydrin test | _ | _ |
| | | Biuret test | _ | _ |
| 5. | Saponins | Foam test | _ | + |
| 6. | Phenolic compunds | $FeCl_3$ test | _ | + |
| | | Gelatin test | _ | + |
| | | Lead acetate test | _ | + |
| 7. | Phytosterol | Salkowiski test | _ | - |
| | | Libermann burchard | _ | - |
| 8. | Alkaloids | Dragendroff's test | _ | + |
| | | Mayer's test | _ | + |
| | | Wagner's test | _ | + |
| | | Hager's test | _ | _ |
| 9. | Gum&mucilage | Swelling test | _ | – |
| 10. | Flavonoids | Aqueous NaOH | _ | + |
| | | Con. $H_2SO_4$ test | _ | + |
| | | Shinoda's test | _ | + |

The phytochemical evaluation shows the presence of flavonoid, phenolic compounds, tannins, glycosides, saponins, and carbohydrate in the petroleum ether and ethanolic extact.

**Oral Acute Toxicity Studies**

**$LD_{50}$ value of 50 % ethanolic extract of *Cinnamomum tamala leaves***

Animals used - mice
Weight of animals - 20-25g
No.of animals per group - 3
Route - oral

**Table no: 3. Oral acute toxicity studies**

| SI. No. | No. of animals per Group | Dose | No. of death of animals |
|---|---|---|---|
| 1 | 3 | 5 mg/kg | 0 |
| 2 | 3 | 50 mg/kg | 0 |
| 3 | 3 | 300 mg/kg | 0 |
| 4 | 3 | 2000 mg/kg | 1 |

$LD_{50}$ value = 2000 mg/kg
$ED_{50}$ value = 200 mg/kg

## *In Vitro* Antidiabetic Studies

**Table No. 4. Observation of 50% ethanolic extract of leaves of *Cinnamomum tamala* on α-amylase inhibition activity**

| Tube | Amylase | Buffer-ph 6.8 | Time until starch disappear (min) |
|---|---|---|---|
| 1 | 1ml tube1 0.5 ml starch solution 2% Alcoholic extract | 20 drops | 16 |
| 2 | 1ml tube2 0.5 ml starch solution 1% Alcoholic extract | 20 drops | 14 |
| 3 | 1ml tube3 0.5 ml starch solution0.5% Alcoholic extract | 20 drops | 13 |
| 4 | 1ml tube4 0.5 ml starch solution 0.25% Alcoholic extract | 20 drops | 11 |

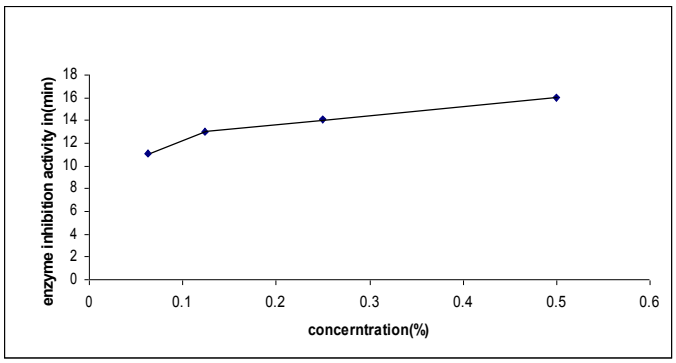



**Fig. No. 1. α Amylase Inhibition Activity of *Cinnamomum Tamala***

**Table No. 5. Anti-diabetic effect (Glucose level) of 50% ethenolic extract of leaves of *Cinnamomum tamala* (ECT) on streptozotocin induced diabetic rats after 0 and 21 days**

| Groups | Treatment | Dose | 0 day (mg/dl) | After 21 days (mg/dl) |
|---|---|---|---|---|
| **I** | **Control normal** | 10 ml/kg | 92.33 ± 5.71 | 92.66 ± 5.88 |
| **II** | **Diabetic control** | 55 mg/kg | 282.66 ± 26.14 | 284.75 ± 25.43 |
| **III** | **Streptozotocin+ ECT 200** | 200 mg/kg | 270.16 ± 24.94[a] | 184.5 ± 16.73[a] |
| **IV** | **Streptozotocin+ ECT 400** | 400 mg/kg | 272.33 ±23.66[a] | 159.83 ± 13.22[c] |
| **V** | **Glibenclamide** | 600 µg/kg | 242.21 ±21.59[a] | 138.16 ± 11.35[c] |
| **One-way ANOVA** | | | | |
| **F** | | | 13.36 | 20.22 |
| **df** | | | 4 | 4 |
| **p** | | | <0.001 | <0.001 |

Value are expressed as Mean ± SEM of 6 rats in each group and 6 rats in Diabetic control.
a =P<.05, b = P < 0.01 ,c = P<.001compared with diabetic control group.

**Table No. 6. Effect of 50% ethenolic extract of leaves of *Cinnamomum tamala* (ECT) on the glucose level, cholesterol, and triglyceride in blood serum**

| Groups | Treatment | Dose | Glucose level (mg/dl) after 21 days | Cholesterol (mg/dl) | Triglyceride (mg/dl) |
|---|---|---|---|---|---|
| I | Control normal | 10 ml/kg | 92.66 ± 5.88 | 72.82 ± 6.01 | 81.25 ± 7.97 |
| II | Diabetic control | 55 mg/kg | 284.75± 25.43 | 103.61±9.43 | 105.79± 9.66 |
| III | Streptozotocin+ ECT 200 | 200 mg/kg | 184.5± 16.73[a] | 82.25± 7.97[a] | 99.95 ± 8.69[a] |
| IV | Streptozotocin+ ECT 400 | 400 mg/kg | 159.83± 13.22[c] | 79.54 ± 8.12[a] | 88.99 ± 7.70[a] |
| V | Glibenclamide | 600 µg/kg | 138.16 ± 11.35[c] | 76.71 ±6.76[c] | 84.01 ± 6.97[c] |
| One-way ANOVA<br>F<br>df<br>p | | | 20.22<br>4<br><0.001 | 15.76<br>4<br><0.001 | 1.623<br>4<br><0.001 |

expressed as Mean ± SEM of 6 rats in each group and 6 rats in Diabetic control
a= P< 0.05, b = P < 0.01 compared with diabetic control group.

**Table No. 7 . Effect of 50% ethanolic extract of leaves of *Cinnamomum tamala* (ECT) on SGOT, SGPT, and SALP level in blood serum**

| Groups | Treatment | Dose | SGOT (U/l) | SGPT (U/l) | SALP (U/l) |
|---|---|---|---|---|---|
| I | Control normal | 10 ml/kg | 63.59 ± 5.55 | 21.28 ± 1.89 | 229.66 ± 21.76 |
| II | Diabetic control | 55 mg/kg | 93.73 ± 8.53 | 49.56± 4.12 | 296.5 ± 24.04 |
| III | Streptozotocin+ ECT 200 | 200 mg/kg | 70.91 ± 6.53[b] | 37.10 ± 2.47[b] | 251.16 ± 22.60[a] |
| IV | Streptozotocin+ ECT 400 | 400 mg/kg | 67.90 ± 5.85[a] | 29.12 ± 2.76[c] | 214.66± 21.55[a] |
| V | Glibenclamide | 600 µg/kg | 65.92 ± 5.33[a] | 23.16 ± 2.87[c] | 202.33 ± 21.71[c] |
| One-way ANOVA<br>F<br>df<br>p | | | 3.574<br>4<br>< 0.001 | 15.76<br>4<br>< 0.001 | 2.741<br>4<br>< 0.001 |

expressed as Mean ± SEM of 6 rats in each group and 6 rats in Diabetic control group.
a= P< 0.05, b = P < 0.01, c = p< .001 compared with diabetic control group.

**Table No. 8. Free radical scavenging activity (FRSA) of 50% ethanolic extract of leaves of *Cinnamomum tamala* (ECT) by the use of DPPH (1, 1-diphenyl-2-picrilhydrazyl) reduction**

| Concentration(µg/ml) | DPPH % inhibition |
|---|---|
| 200 | 69.76 ± 2.37 |
| 400 | 78.39 ± 1.88 |
| 600 | 84.42 ± 1.79 |
| 800 | 90.89 ± 2.07 |

Values are expressed as Mean ± SEM of triplicate of each sample

**Table No. 9 . Effect of 50% ethenolic extract of leaves of *Cinnamomum tamala* (ECT) on lipid peroxidation, superoxide dismutase, catalase and glutathione peroxidase in 21 days**

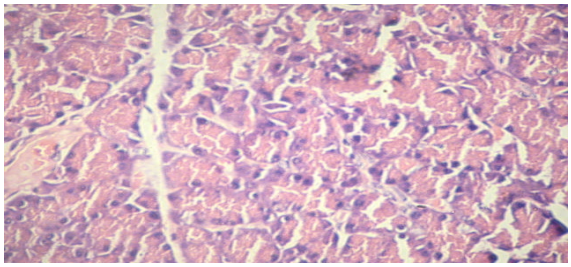
| Groups | Treatment | Dose | LPO (n moles/mg of protein) | SOD (units/mg of protein) | CAT (units/mg of protein) | GPx (m moles/gm) |
|---|---|---|---|---|---|---|
| I | Control normal | 10 ml/kg | 0.31 ± 0.02 | 145.70 ± 13.42 | 44.51 ± 4.21 | 7.05 ± 0.9 |
| II | Diabetic control | 55 mg/kg | 0.86 ± 0.08 | 108.39 ± 10.65 | 17.26 ± 1.10 | 1.95 ± 0.2 |
| III | Streptozotocin+ ECT 200 | 200 mg/kg | 0.42 ± 0.04[a] | 134.18 ± 11.66[a] | 31.51 ± 2.12[b] | 3.66 ± 0.4[a] |
| IV | Streptozotocin+ ECT 400 | 400 mg/kg | 0.39± 0.03[c] | 139.85 ± 11.51[a] | 37.14 ± 3.21[c] | 4.55 ± 0.8[a] |
| V | Glibenclamide | 600 µg/kg | 0.36 ± 0.031[c] | 142.40 ± 12.18[a] | 39.31 ± 3.94[c] | 5.71 ± 0.35[c] |
| One-way ANOVA<br>F<br>df<br>p | | | 24.20<br>4<br>< 0.001 | 1.579<br>4<br><0.001 | 11.04<br>4<br>< 0.001 | 10.68<br>4<br><0.001 |

Expressed as Mean ± SEM of 6 rats in each group and 4 rats in Diabetic control
a= P< 0.05, b = P < 0.01 c = p<.001 compared with diabetic control group

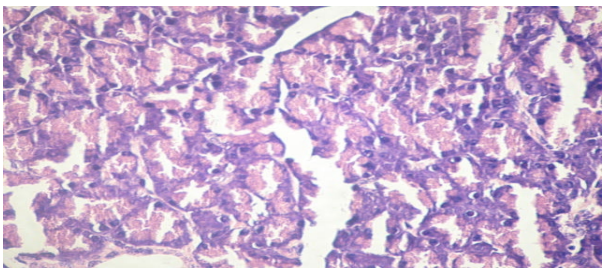
**Histopathological studies**

The normal architecture was restored to the same as that of the standard drug (Glibenclamide) treated kidney (figure no. 3. J). This is evident that 50% ethanolic extract of leaves of *Cinnamomum tamala* posses good antidiabetic and antioxidant activity.

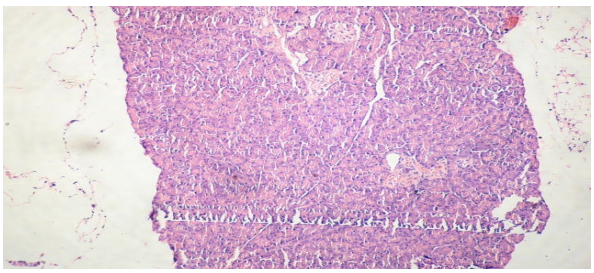
**I) Pancreas**



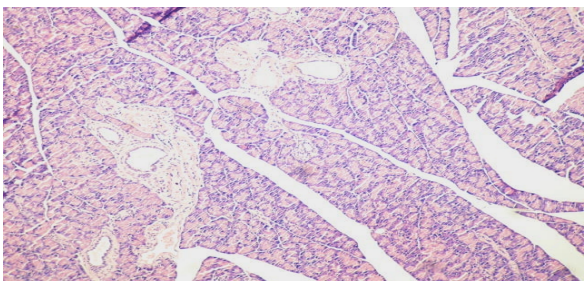
**(A) Normal Control**



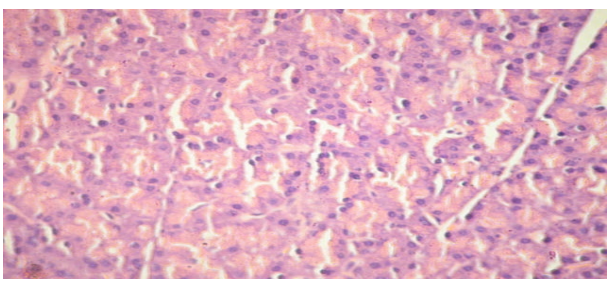
**(B) Diabetic Control**



**(C) Diabetic + ETC 200**



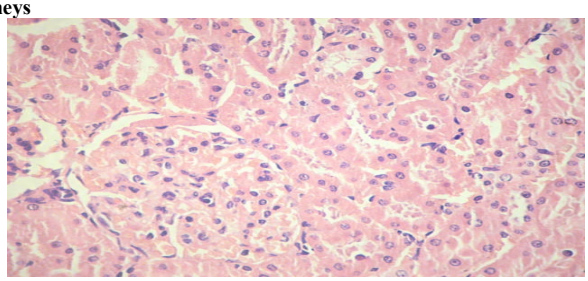
**(D) Diabetic + ECT 400**



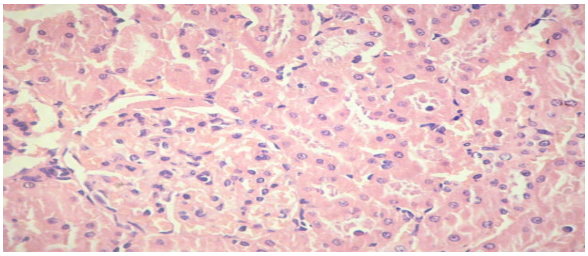
**(E) Diabetic + Standard**

**Fig. No. 2 (A) Normal Control**
**(B) Diabetic Control**
**(C) Diabetic + ETC 200**
**(D) Diabetic + ECT 400**
**(E) Diabetic + Standard**

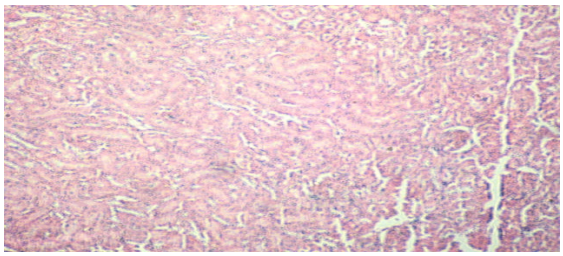
**II) Kidneys**



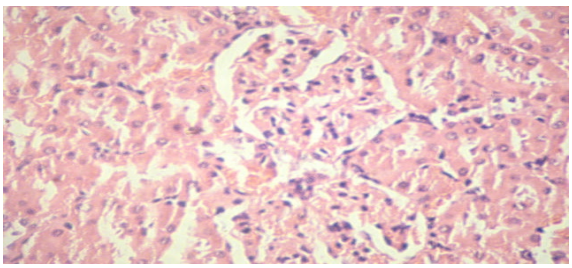
**(F) Normal Control**



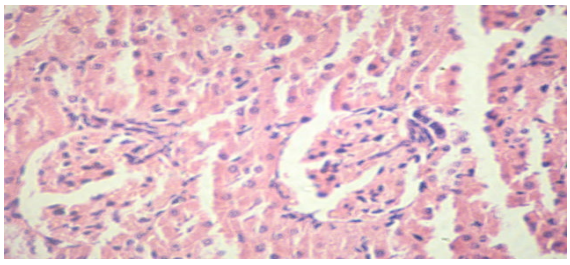
**(G) Diabetic Control**



**(H) Diabetic + ECT 200**



**(I) Diabetic + ECT 400**



**(J) Diabetic + Standard**

**Figure 3 : (F) Normal Control**
**(G) Diabetic Control**
**(H) Diabetic + ECT 200**
**(I) Diabetic + ECT 400**
**(J) Diabetic + Standard**

## CONCLUSION

The present study revealed that the 50% ethanolic extract of leaves of *Cinnamomum tamala* not only possess good antihyperglycemic properties but also have very good antioxidant activity in streptozotocin induced diabetic condition. The 50% ethanolic extract of leaves of *Cinnamomum tamala*, which shows diabetes biology and pharmacology lead to rapid development in diabetes treatment.

In addition to this, studies on molecular aspect of diabetic therapy will give mechanistic information in diabetes therapy and also critical balance should be there between the animal model and clinical research. This holds great promise for future research in human beings.

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