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Anti-hyperglycemic and Anti-oxidant activities of Ethanolic extract of *Lantana camara* Leaves

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Abstract

The present study has been undertaken to evaluate *In-vivo* Anti-hyperglycemic and anti-oxidant activities of ethanolic *Lantana camara* leaves extract (LCE). The extract was prepared by cold percolation process. Phytochemical screening indicates that the presence of flavanoids, phenols, carbohydrates etc. The Anti-hyperglycemic activity of the extract was studied by *In-vivo* using Streptozotocin induced diabetes rat models. Glibenclamide was used as a standard drug to compare the blood glucose level. The groups treated with *Lantana camara* leaves extract at dose 200 and 400 mg/kg prevented the diabetes condition in a dose related manner. LCE and Glibenclamide treated rats showed decreased LPO that is associated with increased activity of SOD and catalase. The increase in the level of lipid peroxides in plasma generally is thought to be the consequence of increased production of and liberation in to the circulation of tissue lipid peroxides due to pathological changes. This action shows the Anti-peroxidative effect of LCE. The results obtained thus suggest that 50% ethanolic *Lantana camara* leaves extract possesses potent Anti-hyperglycemic and Anti-oxidant activity.

Keywords: *Lantana camara*; Ethanol extract; Streptozotocin; Anti-hyperglycemic activity; Anti-oxidant activity.

1. Introduction

Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. It leads to hyperglycemia that in due course turns into a syndrome called Diabetes mellitus [1]. Diabetes mellitus (predominantly type 2 diabetes) is a major and growing health problem in almost all the countries. Globally, the number of people with diabetes will be more than double over the next 25 years, to reach a total of 366 million by 2030 [2]. Medicinal plants and their bioactive constituents are used for the treatment of Diabetes throughout the world. Many indigenous medicinal plants have found to be useful to manage diabetes. The synthetic drugs are either too expensive or have undesirable effects or contraindications. Moreover, as the heterogeneity of this disorder increases, there is need to look for more efficacious agents with lesser side effects [3]. In the present study, attempt was made to prove the anti-hyperglycemic effect of *Lantana camara*. The stem, root and leaves contain many of the bioactive compounds responsible for various therapeutic applications such as cancers, swellings, chicken pox, asthma and curing infections [4].

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2. Material and methods

2.1. Chemicals and Instruments

- Drug for induction of Diabetes: Steptozotocin.
- Standard drug used: Glibenclamide
- All other reagents used were of analytical grade.
- Instruments used: Percolator, UV-Visible Spectrophotometer.

2.2. Plant Material and Extraction Procedure

The leaves of *Lantana camara* (Family – *Verbenaceae*) were collected from Local area, Attur, Salem, Tamilnadu, India (Fig 1). The plant specimen (No: BSI/SRC/5/23/2016/Tech/1211) was authenticated by Dr. M. Palanisamy, Scientist D, In-charge, Botanical Survey of India, Southern Regional Centre, Coimbatore, TamilNadu, India. The dried leaves were ground to coarse powder by grinder. Ethanolic extract of *Lantana camara* was produced by cold percolation. The percentage yield of extract was found to be 9.5 % w/w.



Figure 1. *Lantana camara* Leaves

2.3. Preliminary Phytochemical Analysis

50% ethanolic extract of *Lantana camara* was subjected to qualitative tests for the identification of various active constituents' viz. carbohydrate, glycosides, alkaloids, amino acids, flavanoids, fixed oil, tannins, gum and mucilage, phytosterols etc. according to Khandelwal [5].

3. Pharmacological evaluation

3.1. Animals

Sprague-Dawley rats (150-185 g) and Swiss albino mice (20-25 g) of either sex and of approximately the same age were procured from the animal house of J. K. K. Nattraja College of Pharmacy, Kumarapalayam, Namakkal, Tamilnadu. All animals were maintained under standard laboratory conditions (Temperature: 22±2°C and humidity: 45±5%) with 12 hours day: 12 hours night cycle. 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 [6].

3.2. Acute Toxicity studies (OECD Guideline 423)

3.2.1. Requirements

Animal: Swiss albino mice (female 20-25 g). Crude Extract: 50% ethanolic extracts of *Lantana camara* Leaves.

- The overnight fasted mice were weighed and selected.
- Ethanolic extract was dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce some signs of toxicity and were observed for a period of two weeks (Fig 2).

- The toxic doses were selected based on the Guideline 423.
- The result of the LD₅₀ study was done by mice using guideline 423 method.

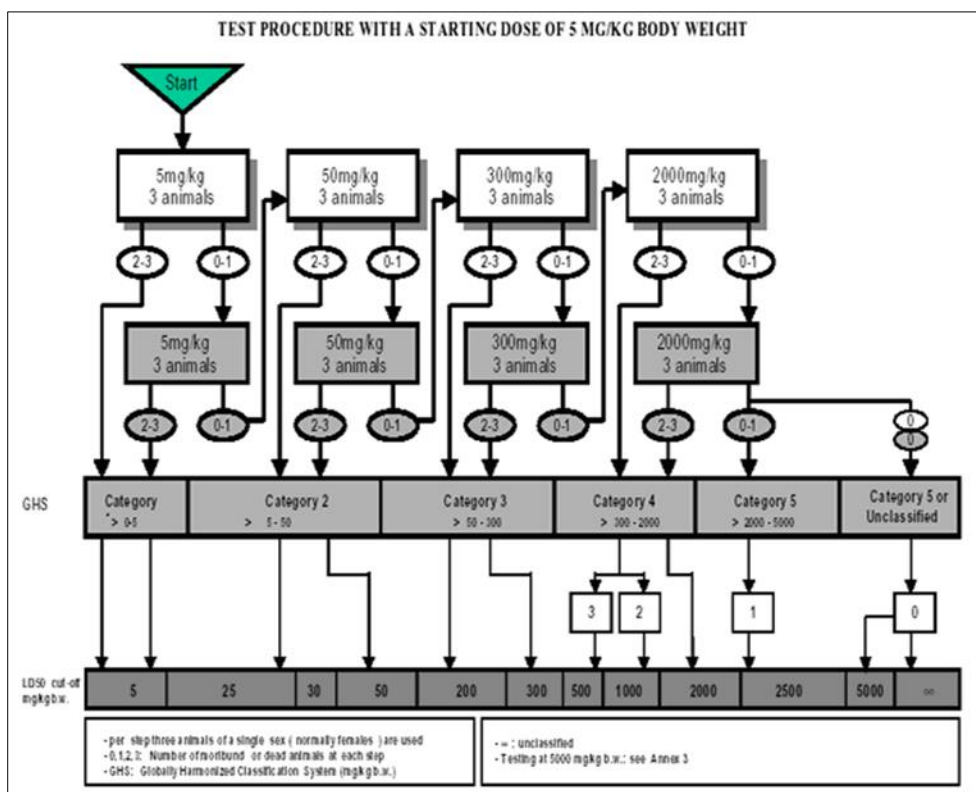


Figure 2. Acute toxicity study

3.3. *In-vivo* Anti-hyperglycemic activity

3.3.1. Streptozocin induced Diabetes Mellitus in models

All animals were allowed to adapt to metabolic cages for 3 days, after which they were fasted overnight and 150 mg/kg Streptozotocin freshly dissolved in normal saline was injected intraperitoneally. Blood glucose levels were measured 3 days after Streptozotocin injection. The animals having a blood glucose level higher than 200 mg/dL was considered diabetic and was used for the experiments [7].

3.4. Evaluation of Anti-hyperglycemic activity of *Lantana camara* leaves in Steptozotocin induced diabetic rats

3.4.1. Experimental design

- Group I – Control rats received vehicle solution (1% CMC).
- Group II – Diabetic control rats received vehicle solution (1% CMC).
- Group III – Diabetic rats treated with extract 200 mg/kg body weight in 1% CMC
- Group IV – Diabetic rats treated with extract 400 mg/kg body weight in 1% CMC
- Group V – Diabetic rats treated with Glibenclamide 5 mg/kg in aqueous solution.

Fresh blood drawn was centrifuged for 10 min at 2000 rpm and the biochemical parameters Cholesterol, Triglycerides, Aspartate Aminotransferase / Serum glutamic oxaloacetic transaminase (ASAT) / (SGOT), Alanine amino transferase / Serum glutamate pyruvate transaminase (ALAT)/(SGPT) and Alkaline phosphatase (ALP) were analysed.

3.5. Bio-chemical Parameters

3.5.1. Total cholesterol estimation

The serum cholesterol level was estimated by Wybenga and Pileggi method using cholesterol diagnostic reagent kit (span). Absorbance was measured at 560 nm.

Procedure

Pipette into tubes marked	Blank	Standard	Test
Reagent 1 : Cholesterol reagent	5.0 ml	5.0 ml	5.0 ml
Reagent 2 : Working cholesterol	-	0.025 ml	-
Standard 200mg % Serum	-	-	0.025 ml

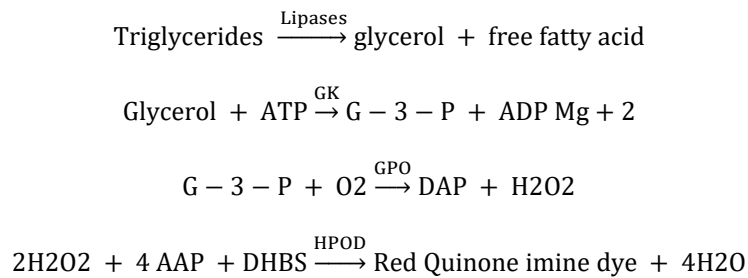
Calculation

$$\text{Total cholesterol (mg/dl)} = \frac{\text{O. D. of test}}{\text{O. D. of standard}} \times 200$$

3.6. Triglycerides estimation

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

3.6.1. Reaction



3.6.2. Procedure

Reagents	Test	Standard	Blank
Working Reagent	1.0 ml	1.0 ml	1.0 ml
Sample	10µl	----	----
Standard	----	10µl	----

3.6.3. Calculation

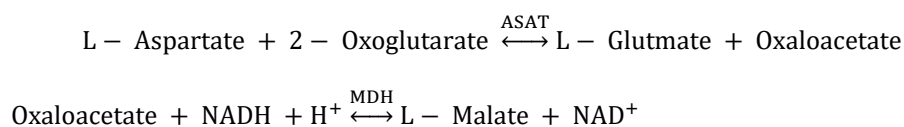
$$\text{Triglyceride conc. (mg / dl)} = \frac{\text{A of Test}}{\text{A of Std}} \times 200$$

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.

3.7. Determination of Serum glutamic oxaloacetic transaminase (SGOT)

The SGOT activity was determined according to the method of IFCC modified method using SGOT (Liquizone diagnostic reagent kit).

3.7.1. Principle



3.7.2. Procedure

Working Reagents	1.0 ml
Serum / Plasma	100 µl

3.7.3. Calculation

Calculate the average change in absorbance per minute (Δ Abs / min).

Activity of ASAT (SGOT) in IU/L.

3.8. Determination of Serum glutamate pyruvate transaminase (SGPT) or ALAT

The SGPT activity was determined according to the method of IFCC modified method using SGPT (Liquizone diagnostic reagent kit).

3.8.1. Procedure

Working Reagents	1.0 ml
Serum / Plasma	100 µl

3.8.2. Calculation

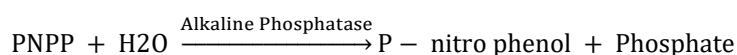
Calculate the average change in absorbance per minute (Δ Abs/min).

Activity of ALAT (SGPT) in IU/L.

At 340 nm in IU/L = Δ Abs/min \times 1746 \times t f

3.9. Determination of serum alkaline phosphatase (SALP)

The alkaline phosphates level was estimated by p- Nitro phenyl phosphate (PNPP) method (Qualigens diagnostic reagent kit).



3.9.1. Procedure

	Test (T)	Blank (B)
Working reagent	1.0 ml	Distilled water
Sample	20 µl	Distilled water

3.9.2. Calculation

IU /L of Alkaline phosphatase = Δ A /min \times 2713

Where F = 2713 is calculated on the basis of molar extinction coefficient for p- nitro phenol and total assay volume to sample volume.

3.10. Evaluation of Anti-oxidant activity of *Lantana camara* in Streptozotocin induced diabetic rats

3.10.1. Assay of lipid per oxidation

The concentration of thiobarbituric acid reactive substances (TBARS) was measured (lipid per oxidation product maondialdehyde (MDA) was estimated) in liver using the method of Okhawa et al. The concentration was expressed as n moles of MDA per mg of protein using 1, 1, 3, 3,-tetra-ethoxypropane as the standard [8].

3.10.2. Catalase activity

CAT activity was determined by monitoring the enzyme-catalyzed decomposition of hydrogen peroxide by potassium permanganate according to Cohen et al. [9]. The measured activities were normalized to the protein content of each sample.

3.10.3. Superoxide dismutase activity

A system devoid of enzymes served as control. One unit of the enzyme activity is defined as the enzyme concentration required to inhibit the optical density at 560 nm of chromogen production by 50 % in one min under the assay conditions and expressed as specific activity in mill units/ mg protein [10].

3.10.4. Glutathione peroxidase

The 50% ethanolic extracts of leaves of *Lantana camara* and Glibenclamide treated rats showed decreased LPO that is associated with increased activity of SOD, catalase and GPx.

3.11. Statistical Analysis

The data expressed as mean \pm SEM was statistically analyzed 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 Comparison Test. The value of probability less than 5% ($P < 0.05$) was considered statistically significant.

4. Results and discussion

4.1. Plant Material and Extraction

The dried leaves of ethanolic extract of *Lantana camara* was produced by cold percolation. The percentage yield of extract was found to be 9.5 % w/w (Table 1).

Table 1 Percentage yield of *Lantana camara* with 50% ethanol

Plant used	Part used	Method	Percentage yield
<i>Lantana camara</i>	Leaves	Percolation with 50% ethanol	9.5% w/w

4.2. Preliminary Phytochemical Analysis

Preliminary Phytochemical Analysis shows the presence of flavonoids, glycosides carbohydrates, alkaloids, tannins, phenolic compounds, steroids, terpenoids and amino acids (Table 2).

Table 2 Preliminary phytochemical screening of the 50 % ethanolic extract of *Lantana camara*

S. No.	Constituents	Tests	50% Ethanolic extract
1.	Carbohydrate & Glycosides	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 compounds	FeCl ₃ test	+
		Gelatin test	-

		Lead acetate test	+
7.	Phytosterol	Salkowski test	+
		Libermann burchard test	+
8.	Alkaloids	Dragendroff's test	+
		Mayer's test	+
		Wagner's test	+
		Hager's test	-
9.	Gum & mucilage	Swelling test	-
10.	Flavonoids	Aqueous NaOH test	+
		Con. H ₂ SO ₄ test	+
		Shinoda's test	+

4.3. Pharmacological Evaluation

4.3.1. General behavior and acute toxicity studies

50% ethanolic extract of selected plant *Lantana camara* Leaves upto 2000 mg/kg did not cause any mortality in mice. None of the doses tested produced any gross apparent effect on general motor activity, muscular weakness, fecal output, feeding behavior etc. during the period of observation.

4.4. Effect of 50% ethanolic extract of *Lantana camara* on Streptozotocin induced diabetes

4.4.1. Streptozotocin induced diabetic rats after 0 day and 21 days

Streptozotocin induced the significant increase in the blood glucose level at 0 day (72.33-292.66, $p < 0.0001$). The 50% ethanolic extract of the *Lantana camara* showed the significant effect compared with the respective diabetic control group, decrease the blood glucose level at a dose of 200 mg/kg and 400 mg/kg (274.75-205.05, 274.75-199.83, $p < 0.0001$), the standard drug Glibenclamide 5 mg/kg also showed the significant decrease the blood glucose level after 21 days (274.75-162.16, $p < 0.0001$). Finally the 400 mg/kg and the standard drug showed the significant decrease in the blood glucose level after 21 days treatment ($p < 0.0001$) as given in (Table 3).

Table 3 Streptozotocin induced diabetic rats after 0 day and 21 days

Group	0 day (mg/dl)	After 21 days (mg/dl)
Control	72.33±0.71	72.66 ± 0.88
Diabetic control	292.66 ± 1.14	274.75± 1.43
LCE (200 mg/kg)	280.16 ±0.94	205.5 ^b ± 1.73
LCE (400 mg/kg)	272.33± 0.66	199.83± 1.22
Glibenclamide (5 mg/kg)	252.00 ± 1.59	162.16 ± 1.35
One-way ANOVA		
F	7,411	2,739
df	4	4
p	<0.0001	<0.0001

Values are expressed as Mean ± SEM of 6 rats in each group and 4 rats in diabetic control group
b = P < 0.01 – (LCE 400 mg/kg Vs. LCE 200 mg/kg)

4.5. Effects of 50% ethanolic extract of *Lantana camara* on the blood glucose Levels of Streptozotocin induced diabetic rats

The blood glucose levels of diabetic rats treated with LCE at doses of 200 and 400 mg/kg showed significant differences at 2, 3 and 4 h from initial levels ($P < 0.0001$). Only diabetic rats with blood glucose level of 252.0 ± 1.59 mg/dl showed a significant and pronounced reduction of glycemia ($P < 0.0001$) 2, 3 and 4 hr after oral Glibenclamide (5 mg/kg) administration. The LCE was found to be slow and less effective than Glibenclamide in diabetic rats as given in (Table 4).

Table 4 Blood glucose Levels of Streptozotocin induced diabetic rats

Group	0 hr (mg/dl)	2 hr (mg/dl)	3 hr (mg/dl)	4 hr (mg/dl)
Normal control	72.33±0.71	71.83 ± 0.94	72.16 ± 0.40	71.66 ± 0.55
LCE(200 mg/kg)	280.16 ± 0.94	276.0 ± 0.57	274.66 ± 0.71	270.16 ± 0.75
LCE(400 mg/kg)	272.33 ± 0.66	267.83 ± 0.60	264.66 ± 0.80	260.83 ± 0.91
Glibenclamide (5mg/kg)	252.0 ± 1.59	248.16 ± 0.98	246.16 ± 0.75	241.66 ± 1.28
One-way ANOVA				
f	8,943	14,820	19,570	10,540
df	3	3	3	3
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values are expressed as Mean ± SEM of 6 rats in each group

4.6. Effect of 50% ethanolic extract of *Lantana camara* on Cholesterol and Triglyceride, in Streptozotocin induced diabetic rat serum

It is clearly evident that Streptozotocin caused significant elevation of serum markers. The STZ treated group, the level of cholesterol (72.82-103.61, $p < 0.0001$), triglyceride (81.25-105.79, $p < 0.0001$). In contrast, the groups treated with *Lantana camara* Leaves extract at dose 200 and 400 mg/kg once daily for 21 days prevented the diabetes condition in a dose related manner. The range of protection were found to be, Cholesterol (103.61-79.54, $p < 0.05$, $p < 0.0001$), Triglyceride (105.79-84.01, $p < 0.05$, $p < 0.0001$) and Glibenclamide (5 mg/kg) also showed the significantly decrease with respect to diabetic control group (103.61-76.71, $p < 0.0001$, $p < 0.01$, 105.79-84.01, $p < 0.0001$, $p < 0.05$) (Table 5).

Table 5 Cholesterol and Triglyceride, in Streptozotocin induced diabetic rat serum

Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Control	72.82 ± 1.01	81.25 ± 0.97
Diabetic control	103.61±0.43	105.79± 1.66
LCE (200 mg/kg)	82.25 ^a ± 0.97	99.95 ± 0.69
LCE (400 mg/kg)	79.54 ^a ± 0.94	88.99 ± 0.70
Glibenclamide (5 mg/kg)	76.71 ^b ± 0.76	84.01 ^a ± 0.97
One-way ANOVA		
F	140.9	109.9
df	4	4
p	<0.0001	<0.0001

Values are expressed as Mean ± SEM of 6 rats in each group and 4 rats in diabetic control group.

a= $P < 0.05$ - (Std. Vs. LCE 400, LCE 400 Vs. LCE 200, Cont. Vs. Std);

b = $P < 0.01$ - (Cont. Vs. Std)

4.7. Effect of 50% ethanolic extract of *Lantana camara* on SGOT, SGPT and SALP

STZ induced a significant increase in SGOT (63.59–73.73, $p < 0.01$, $p < 0.0001$), SGPT (21.28–29.56, $p < 0.05$, $p < 0.0001$) and SALP (229.66–261.5, $p < 0.0001$). The 50% ethanolic extracts of 200, and 400 mg/kg of *Lantana camara* decreases the SGOT (73.73–67.90, $p < 0.0001$, $p < 0.01$, $p < 0.05$), SGPT (29.56–25.12, $p < 0.0001$, $p < 0.05$) and SALP (261.5–234.66, $p < 0.05$, $p < 0.0001$) levels with respect to diabetic control group and Glibenclamide (5 mg/kg) also showed significant decrease when compared to diabetic control group. (73.73–65.92, 29.56–23.16, 261.5–232.33, $p < 0.0001$, $p < 0.05$ (Table 6).

Table 6 Effect of 50% ethanolic extract of *Lantana camara* on SGOT, SGPT and SALP

Oral treatment (mg/kg, OD x 21 days)	SGOT (U/l)	SGPT (U/l)	SALP (U/l)
Control	63.59 ± 0.55	21.28 ± 0.29	229.66 ± 0.76
Diabetic control	73.73b ± 0.53	29.56a ± 0.79	261.5 ± 1.04
LCE (200 mg/kg)	70.91b ± 0.53	27.10 ± 0.47	237.16a ± 0.60
LCE (400 mg/kg)	67.90a ± 0.85	25.12a ± 0.76	234.66a ± 0.55
Glibenclamide (5 mg/kg)	65.92a ± 0.33	23.16a ± 0.87	232.33a ± 0.71
One-way ANOVA			
F	39.45	22.17	253.8
df	4	4	4
p	< 0.0001	< 0.0001	< 0.0001

Values are expressed as Mean ± SEM of 6 rats in each group and 4 rats in diabetic control group.

a = $P < 0.05$ - (Cont. Vs. Std., Std. Vs. LCE 400), (Cont. Vs. Std., Std. Vs. LCE 400, LCE 400 Vs. LCE 200, LCE 200 Vs. Dia. Control.), (Cont. Vs. Std., Std. Vs. LCE 400, LCE 400 Vs. LCE 200)

b = $P < 0.01$ - (LCE 400 Vs. LCE 200, LCE 200 Vs. Dia. Control)

The differences in mean SGOT, SGPT, SALP, in extract treated groups are not significantly different from control group at the end of study (21 days).

4.8. Effect of 50% ethanolic extract of *Lantana camara* on LPO, CAT, SOD, and GPx against the Streptozotocin induced diabetic rats

Table 7 Effect of 50% ethanolic extract of *Lantana camara* on LPO, CAT, SOD, and GPx against the Streptozotocin induced diabetic rats

Oral treatment	LPO (n moles/mg of protein)	SOD (units/mg of protein)	CAT (units/mg of protein)	GPx (m moles /gm)
Control	0.31 ± 0.006	145.70 ^b ± 0.42	44.51 ± 0.81	7.05 ± 0.09
Diabetic control	0.46 ^b ± 0.008	128.39 ± 1.65	27.26 ± 1.10	2.95 ± 0.07
LCE (200 mg/kg)	0.42 ^a ± 0.014	134.18 ± 0.66	31.51 ^b ± 1.12	3.66 ^b ± 0.07
LCE (400 mg/kg)	0.39 ^a ± 0.009	139.85 ± 0.51	35.14 ^a ± 0.83	4.55 ± 0.08
Glibenclamide (5 mg/kg)	0.36 ± 0.004	142.40 ^a ± 0.88	39.31 ^b ± 0.94	5.71 ± 0.25
One-way ANOVA				
F	36.42	65.27	44.97	129.9
df	4	4	4	4
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values are expressed as Mean ± SEM of 6 rats in each group and 4 rats in diabetic control group.

a = $P < 0.05$ - (Std. Vs. LCE 400, LCE 400 Vs. LCE 200), (LCE 400 Vs. Std.), (LCE 200 Vs. LCE 400)

b = $P < 0.01$ - (LCE 200 Vs. Dia. control), (Std. Vs. Control), (Dia. control Vs. LCE 200, LCE 400 Vs. Std.), (Dia. control Vs. LCE 200)

Streptozotocin caused significant elevation in LPO (0.31-0.46, $p < 0.001$) compared with respective control group, the 50% ethanolic extract of *Lantana camara* decrease the LPO level (0.46-0.39, $p < 0.01$, $p < 0.05$, $p < 0.001$) and standard drug Glibenclamide decrease significantly the LPO level (0.46-0.36). The SOD, CAT, GPx significantly decreased by Streptozotocin (145.70-128.39, 44.57-27.26, 7.05-2.97), 50% ethanolic extract of *Lantana camara* increased the level of SOD (128.39-139.85, $p < 0.0001$), CAT (27.26-35.14, $p < 0.01$, $p < 0.0001$), GPx (2.95-4.55, $p < 0.01$, $p < 0.001$). Standard drug Glibenclamide increased significantly SOD (128.39-142.40, $p < 0.001$), CAT (27.26-39.31, $p < 0.001$), GPx (2.95-5.71, $p < 0.001$) (Table 7).

5. Discussion

Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. Plant drugs are frequently considered to be less toxic and freer from side effects than synthetic ones [11]. Streptozotocin induced diabetes cause an increase in blood glucose level in rats [12]. Our studies show that oral administration of 50 % ethanolic *Lantana camara* Leaves extract decreases blood glucose in diabetic rats. The increase in the level of lipid peroxides in plasma generally is thought to be the consequence of increased production of and liberation in to the circulation of tissue lipid peroxides due to pathological changes [13]. This action shows the Anti-per oxidative effect of LCE. Changes in the levels of antioxidants are observed in diabetic conditions [14].

The present study demonstrated that the 50% ethanolic *Lantana camara* Leaves extract had an anti-hyperglycemic effect in the Streptozotocin induced diabetic rats when administered orally. Administration of LCE and Glibenclamide increased the activities of GPx and GST in diabetic conditions. SOD and catalase are two major scavenging enzymes that remove the toxic-free radical in vivo. LCE and Glibenclamide treated rats showed decreased LPO that is associated with increased activity of SOD and catalase [15]. The results obtained thus suggest that 50% ethanolic *Lantana camara* Leaves extract possesses potent anti-hyperglycemic and anti-oxidant activity.

6. Conclusion

It is hoped that the activity-guided isolation of the extract of this plant may yield valuable therapeutic compound(s) useful for developing powerful hypoglycemic or anti-oxidant drugs. The study also demonstrates that pharmacological screening based on the ethno medical leads can yield faster hits in search of therapeutic agents from plants.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

All applicable International, National and /or Institutional guidelines for the care and use of animals were followed.

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