



ANTIDIABETIC ACTIVITY OF DIFFERENT LEAF EXTRACTS OF *CASSIA TORA* IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

In traditional medicine leaves of *Cassia tora* (Leguminosae) are used as laxative, antifungal and antibacterial activity. Some biological reports on seeds revealed, it has antidiabetic activity. Present study was carried out to strengthen antidiabetic activity of *Cassia tora* leaves. Leaf powder was extracted with petroleum ether, chloroform, ethyl acetate, methanol and water successively by maceration. Phytochemical screening of those extracts was carried out. And found to contain amino acids, flavonoids, steroids, alkaloids, saponins, tannins and glycosides. All extracts Petroleum ether extract (PE), Chloroform extract (CE), Ethyl acetate extract (EE), Methanol Extract (ME), and Aqueous extract (AE) were screened for antioxidant activity by Reducing power method and Hydrogen peroxide method. Among all the extracts, ethyl acetate extract exhibited strong activity, Methanol and Aqueous extracts gave moderate activity which is also comparable with Ascorbic acid. Hence EE, ME and AE were screened for antidiabetic activity by alloxan induced method. Glibenclamide was used as standard drug (mg/kg b. w.) EE, ME and AE showed significant activity in the dose dependent manner comparable with Standard. All the three extracts ethyl acetate, methanol and aqueous extracts were found to increase the glucose tolerance in the order EE > ME > AE.

KEY WORDS

Diabetes mellitus, *Cassia tora*, alloxan, oral blood glucose.

INTRODUCTION:

Diabetes mellitus (DM) a leading metabolic disorder worldwide, characterized by hyperglycemia associated with impairment in insulin secretion and/or insulin action as well as alteration in intermediary metabolism of carbohydrate, protein and lipids. Several reports indicate that annual incidence rate of diabetes mellitus will increase in future worldwide, especially in India. Diabetes mellitus is a chronic disorder characterized by high blood glucose levels due absolute or relative deficiency of circulating insulin levels [1].

Diabetes mellitus is a worldwide health problem afflicting millions in both developed and developing countries. It is the prime cause of chronic kidney failure,

blindness, high blood pressure and premature coronary artery diseases [2].

In the recent years emphasis is on the development of drug from plants or the treatment of various diseases including diabetes mellitus, the incidence of which is very high all over the world. The reason is that plant drugs could be effective and at the same time have less or no side effects [3,4].

Cassia tora is a medicinal plant with common name chakramarda. It is distributed throughout the world and used in ayurvedic preparations and it is excellent medicine for skin diseases.

Butanol fraction from *Cassia tora* seeds have glycemic control and insulin secretion activity in diabetic rats was reported in 2008[5]. Anthraquinones from *Cassia tora*

seeds with inhibitory activity on protein glycation and aldose reductase [6].

Hypolipidemic activity of ethanolic extract of seeds of *Cassia tora* was reported in 2004[7]. Seeds are reported to possess hypotensive [8] and hepatoprotective activity [9,10].

Cassia tora is used as laxative [11], oxytocic [12], insecticidal [13], antimutagenic [14], anti-inflammatory [15], antidysentery[16], anthelmintic[17], estrogenic[18], antifungal[19], antibacterial and *Cassia tora* mucilage is also used as suspending agent[20].

Procurement of Plant Materials

For the present investigation, the plant *Cassia tora* was collected from Station Ghanpur, after proper identification by an expert taxonomist, Dr. V.S. Raju, Department of Botany, Kakatiya University, Warangal. The collected plant material was thoroughly checked for foreign organic matter and investigated in terms of invitro antioxidant studies [21]. For preparation of crude drug powder, leaves were taken separately and dried under shade. The completely shade dried leaves were powdered and used for isolation with different solvents (Petroleum ether, Chloroform, Ethyl acetate, Methanol, and Water) by maceration and phytochemical investigations were carried out using leaf powder.

MATERIALS AND METHODS:

Alloxan and Glibenclamide were procured from Sigma-Aldrich Company (St. Louis, Missouri, USA). Ascorbic acid was procured from universal laboratories, Mumbai. H_2O_2 was procured from S.S.Pharm, Hanamkonda. Glucose kit was procured from Taidoc Technology Corporation, San-Chung, Taipei country, Taiwan. The UV spectra were recorded on spectrometer by Shimadzu UV 1700 model. All the solvents and other chemicals were procured from E. Merck, Mumbai and they were of analytical grade quality.

I. IN VITRO ANTIOXIDANT ACTIVITY.

In the present investigation, the petroleum ether, chloroform, ethyl acetate, methanol, and aqueous

extracts of *Cassia tora* leaves were studied for (Invitro) free radical scavenging activity [21,23] of different extracts with Reducing power and Hydrogen peroxide methods.

1.1. Reducing power method:

This method is based on the principle of increase in the absorbance of the reaction mixture indicates increase in the antioxidant activity. In this method antioxidant compound forms a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700nm. Increase in the absorbance of the reaction mixture indicates the reducing power of the samples. Different concentrations of extracts (100µg/ml-1000µg/ml) in 1ml of water were mixed with 2.5ml of phosphate buffer and 2.5ml of potassium ferricyanide. The mixture was incubated at 50°C for 20min, 2.5ml of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000rpm for 10min. The upper layer of the solvent (2.5ml) was mixed with 2.5ml distilled water and 0.5ml of $FeCl_3$ solution and the absorbance was measured at 700nm. Ascorbic acid was used as positive control [24,26].

1.2. Hydrogen peroxide method:

A modified method based on that Ruch et al, was used to determine the ability of the extracts to scavenge hydrogen peroxide. The scavenging of hydrogen peroxide by ascorbic acid and prepared extracts after incubation for 10min increased with increasing concentration and the absorbance was determined at 230nm. Hydrogen peroxide (43Mm) was prepared in phosphate buffered saline (PH 7.4). Positive control (ascorbic acid) and extract solutions were prepared at concentrations of 50 to 250mM. Aliquots of standard or extract solutions (3.4mL) were added to 0.6mL of hydrogen peroxide solution. The reaction mixture was incubated at room temperature for 10min, and the absorbance was determined at 230nm [27].

The percentage of scavenging was calculated as follows

$$\% H_2O_2 \text{ scavenging} = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100.$$

Where A_{control} is the absorbance of control and A_{sample} is the absorbance of the sample.

2. IN VIVO SCREENING OF THE DIFFERENT LEAF EXTRACTS OF CASSIA TORA FOR ANTIDIABETIC ACTIVITY USING ALLOXAN INDUCED MODEL

Materials

- Healthy albino wistar rats of either sex weighing 180-230 gm
- Alloxan monohydrate
- Glibenclamide (Standard drug)

- Different leaf extracts of *Cassia tora*
- Glucometer kit
- Capillary tubes
- Collection tubes

Maintenance of animals:

Albino wistar rats and mice were taken from the Animal Ethical Committee. The animals were acclimatized to the conditions by maintaining them at the experimental conditions for about 7 days prior to dosing. Cage number and individual marking on the tail work made with a marker per to identify the animals. The animals were housed three per cage of same sex in polypropylene cages provided with bedding of paddy. Pellet chew feed standard diet under good management conditions and water libitum was provided to the animals. The temperature 20 – 25° C and 12 hour each at dark and light cycle was maintained.

Induction of diabetes:

Albino rats (180-230 g) of either sex were used for the induction of diabetes. These animals were allowed to fast 18 hours and were injected with freshly prepared aqueous solution of alloxan monohydrate in a dose of 120mg/kg body weight through intra peritoneal route.

Principle:

Glucose + H₂O $\xrightarrow{\text{glucose oxidase}}$ Gluconic Acid + H₂O₂

H₂O₂ + 4-amino antipyrine + p-Hydroxy benzoate \longrightarrow Quinonedye.

Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxidase catalyzed reaction, p-hydroxy benzoate and 4-amino antipyrine react with hydrogen peroxide to form red colored quinine complex. Absorbance data measured at 510nm are directly proportional to glucose concentrations.

Glucose Kit Constitutes Following Reagents (BM):

Glucose reagent \rightarrow glucose oxidase peroxidase

Glucose diluent \rightarrow Phosphate buffer, PH 7.4, phenol.

Glucose standard \rightarrow dextrose (100mg/dl).

Then 5% dextrose was administrated to combat the immediate hypoglycemia. Blood sugar was measured after 18 hours of administration of alloxan. Rats with fasting blood sugar levels around 200 to 400 mg/dl were selected for the study.

Collection of Blood sample:

Blood samples are collected from the retro-orbital plexus of rats, by inserting gently in the inner angle of the eye the capillary is slid under the eye ball at 45° angle and over the bony socket to rupture the fragile venous capillaries of the ophthalmic venous plexus. The passage is about 10mm the tip of the capillary is slightly retracted and the blood was collected in a tube from orbital cavity with the capillary. Capillary tube was held gently merely resting on fingers while blood is flowing. After collecting the desired volume, capillary is removed with simultaneous release of pressure by fore finger and thumb. Any residual blood droplet around the eyeball is wiped off by dry cotton wool.

Analysis of blood samples:

There are several methods for the estimation of blood glucose. In the present study the blood glucose level was determined by used enzymatic, glucose-oxidase-peroxidase (GOD-POD) Method.

Preparation of working reagents

Dissolved the contents, glucose reagents with glucose diluent swirl the mixture gently to dissolve the contents, do not shake vigorously.

Specimen

After collection of the blood sample, it was allowed to clot with slight stirring, with a smooth glass rod, and then the serum is separated by centrifugation at 1500-2000rpm, for 15-20 min. Then the serum glucose is estimated by making dilutions.

	Blank	Standard	Sample
Working	1ml	1ml	0.01ml
Distilled	1 ml	0 ml	0 ml
Standard	*	0.01ml	*
Sample	*	*	0.01ml

Mix well incubates at 37°C for 10min or 20-25 °C for 30min read the optical density at 510nm.

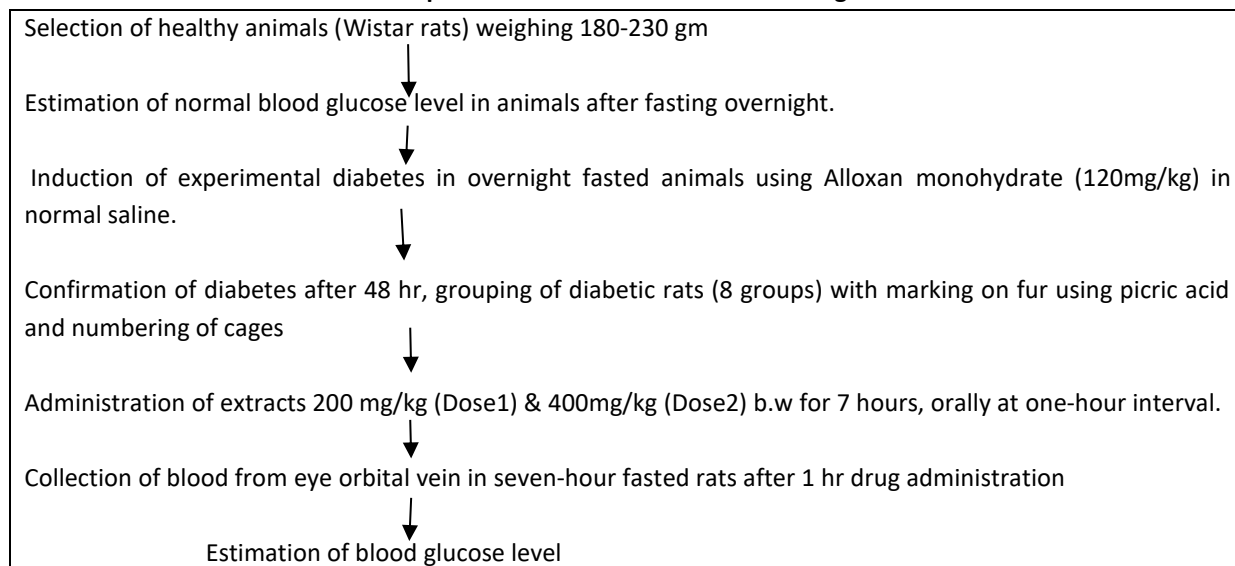
Calculations:

Glucose concentration (mg/dl) = (Absorbance of test/ Absorbance of std.) X100.

Percentage blood glucose reduction= (X_{di}-X_{df}/X_{di}) X 100.

Where X_{di} = Blood glucose level, before the drug treatment; X_{df} = Blood glucose level, after the drug treatment.

Schematic representation of antidiabetic screening of extracts



Experimental Design:

Normal wistar rats of either sex (180 – 230g) were used in the present study. Animals were provided with standard diet and water *ad libitum*. All the rats were fasted for 18 hours prior to the experiments, with only access to water. The rats were divided into different groups containing 6 each. The animals were injected with freshly prepared aqueous solution of Alloxan monohydrate at a dose of 120mg/kg through intraperitoneal route. Then 5% dextrose was administered to combat the immediate hypoglycemia. Blood sugar was measured after 48 hours of induction. Blood glucose levels were determined immediately prior to dosing and 0th, 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th hour after dosing. Blood glucose content was estimated by glucose oxidase method. The hypoglycemic response was calculated by taking the difference in glucose levels at the 0th and subsequent hour. All data, expressed as mean \pm S.D., were statistically analyzed by ANOVA.

Materials:

- Healthy albino wistar rats of either sex weighing 180-230 gm
- Alloxan monohydrate
- Glibenclamide (Standard drug)
- Glucometer kit
- Different extracts of *Cassia tora*.

Maintenance of animals:

Wistar rats and mice were taken from the Animal Ethical Committee. Animals were acclimatized to the conditions by maintaining them at the experimental conditions for about 7 days prior to dosing. Cage number and individual marking on the tail work made with a marker to identify the animals. The animals were housed three per cage of same sex in polypropylene cages provided with bedding of paddy. Pellet chew feed standard diet under good management conditions and water *ad libitum* was provided to the animals. The temperature 20 – 25^o C and 12 hour each at dark and light cycle was maintained.

Administration of glucose:

Overnight fasted rats were divided into groups. (n=3)

Table 1: Showing groups of animals treated with different extracts of *Cassia tora* before the administration of glucose.

Group	Treated
Group I	Control, administered vehicle (gum acacia).
Group II	Diabetic control, treated Alloxan i.p at a dose of 120 mg/kg b.w.
Group III	Standard, glibenclamide at an oral dose of 600 μ g/kg b.w.
Group IV	Methanol extract (ME) of <i>C. tora</i> leaf at oral dose of 200 mg/kg b.w.
Group V	Ethyl acetate extract (EE) of <i>C. tora</i> leaf at oral dose of 200 mg/kg b.w.

Group VI	Aqueous extract (AE) of <i>C. tora</i> leaf at oral dose of 200 mg/kg b.w.
Group VII	Methanol extract (ME) of <i>C. tora</i> leaf at oral dose of 400 mg/kg b.w.
Group VIII	Ethyl acetate extract (EE) of <i>C. tora</i> leaf at oral dose of 400 mg/kg b.w.
Group IX	Aqueous extract (AE) of <i>C. tora</i> leaf at oral dose of 400 mg/kg b.w.

The rats of all the groups were loaded with glucose (2.5gm/kg b.w.) And 30min after the administration of the extracts blood samples were collected at 30, 60, 90 and 120 min after glucose loading. Blood glucose levels were measured immediately.

Collection of Blood sample:

Blood samples are collected from the tail vein, by incising with a scalpel blade a drop of blood was placed on the glucostrip to determine the blood glucose level by using commercially available glucometer kit. After collecting the desired volume of blood, any residual blood droplet around the tail tip is wiped off by dry cotton wool.

Analysis of blood samples:

There are several methods for the estimation of blood glucose. In the present study the blood glucose level was determined by using commercially available glucometer kit.

Test principle:

The test is based on the measurement of electrical current caused by the reaction of glucose with the reagent of the strip. The meter measures this current and displays the corresponding blood glucose level. The strength of the current produced by the reaction depends on the amount of glucose in the blood sample.

Chemical components in sensor:

1. Glucose oxidase (A niger) 30 IU
2. Electron shuttle 1.5 mg
3. Enzyme protector 0.13 mg
4. Non-reactive ingredients 2.5 mg

5. Vial cap 3.0 molecule sieve.

After overnight fasting, a zero-min blood sample (0.2ml) was taken from the rats in normal, diabetic control, normal and plant extract treated group, diabetic and glibenclamide treated groups by orbital sinus puncture. Glucose solution (2.5g/kg) was administered orally immediately. Four more samples were taken at 30, 60, 90, and 120min after glucose administration. Blood glucose content was estimated by glucose oxidase method using commercially available glucometer kit. The hypoglycemic response was calculated by taking the difference in glucose levels at the 0 and subsequent hour. All data, expressed as mean \pm S.D., were statistically analyzed by ANOVA.

RESULTS AND DISCUSSIONS:

I. Qualitative phytochemical screening:

A systematic and complete study of crude drugs includes a thorough investigation of both primary and secondary metabolites derived as a result of plant metabolism. Different qualitative chemical tests were performed to investigate the phytochemical profile of given extracts.

In the present investigation, five extracts were prepared from leaves and each of these extracts were subjected to phytochemical evaluation. Standard tests and reagents were employed to detect various phytochemical constituents. The experimental observations were recorded in the table.

Table 2: Results of Phytochemical evaluation of *Cassia tora* leaf extract

Plant constituents of leaf	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Amino acids	-	-	+	+	-
Flavonoids	-	-	+	+	+
Steroids	+	+	-	-	-
Alkaloids	-	-	+	+	-
Saponins	-	-	+	+	+
Tannins	-	-	+	+	-
Glycosides	-	-	+	+	+

(+): Positive Response; (-): No Response

Some of these results agreed with the previous reports which showed the presence of some amino acids,

flavonoids, steroids, alkaloids, saponins, tannins and anthraquinone glycosides in the leaves of *Cassia tora*.

2. In vitro antioxidant activity:

2.1. Reducing power method:

The reducing capability of the sample extracts was measured by the transformation of Fe^{3+} to Fe^{2+} in the

presence of the extracts at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Table 3: Showing absorbance at 700nm with different leaf extracts of *Cassia tor* by Reducing Power Method

Sl.no	% absorbance at 700nm of different extracts					
Concentration (µg/ml)	Pet.ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract	Ascorbic acid
100	0.12	0.09	0.16	0.11	0.13	0.19
200	0.19	0.13	0.25	0.22	0.21	0.36
400	0.28	0.19	0.31	0.30	0.31	0.48
600	0.33	0.28	0.50	0.46	0.43	0.59
800	0.52	0.32	0.72	0.65	0.68	0.76
1000	0.72	0.50	0.90	0.83	0.81	0.93

Table 4: Results showing % Inhibition of different leaf extracts of *Cassia tora* by Reducing power method.

Sl.no	% Inhibition of different extracts of <i>Cassia tora</i>					
Concentration (µg/ml)	Pet ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract	Ascorbic acid
100	13.25	18.0	17.06	16.43	16.04	20.43
200	26.50	26.0	27.77	26.02	25.92	38.70
400	36.14	38.0	34.44	38.35	38.27	51.61
600	55.42	56.0	55.55	45.20	53.08	63.44
800	78.31	64.2	80.12	71.23	83.95	81.72
1000	100	100	100	100	100	100

Table 5: Results showing IC₅₀ Values of different extracts of *Cassia tora*.

Sl.no	PARTICULARS	IC ₅₀ VALUES(µg/ml)
1	Ascorbic acid	472.5
2	Petroleum ether extract	509.1
3	Chloroform extract	533.3
4	Ethyl acetate extract	505.5
5	Methanol extract	535.4
6	Aqueous extract	500.05

The absorbance of PE, CE, EE, ME and AE of *Cassia tora* was found to be 0.72, 0.50, 0.90, 0.83 and 0.81 respectively and are comparable to standard ascorbic acid whose absorbance was found to be 0.93.

The IC₅₀ values of the PE, CE, EE, ME and AE *Cassia tora* was found to be 509.5, 533.3, 505.5, 535.4 and 500.05 respectively and are comparable to standard ascorbic acid whose IC₅₀ value was found to be 472.5.

2.2. Hydrogen peroxide method:

A modified method based on that Ruch et al. was used to determine the ability of the extracts to scavenge

hydrogen peroxide. Hydrogen peroxide (43Mm) was prepared in phosphate buffered saline (PH 7.4). Positive control (ascorbic acid) and extract solutions were prepared at concentrations of 50 to 250mM. Aliquots of standard or extract solutions (3.4mL) were added to 0.6mL of hydrogen peroxide solution. The reaction mixture was incubated at room temperature for 10min, and the absorbance was determined at 230nm. The percentage of scavenging was calculated as follows:

$$\% H_2O_2 \text{ Scavenging} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100.$$

Where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test.

Table 6: Showing absorbance at 230nm with different extracts of *C. tora* by Hydrogen Peroxide method.

Sl.no	% absorbance at 230nm of different extracts					
Concentration (µg/ml)	Pet ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract	Std Ascorbic acid
50	0.110	0.098	0.126	0.118	0.116	0.135
100	0.276	0.256	0.310	0.296	0.280	0.325
150	0.380	0.372	0.490	0.482	0.398	0.520
200	0.632	0.452	0.743	0.706	0.648	0.753
250	0.710	0.596	0.893	0.858	0.820	0.958

Table 7: Showing % Inhibition of different extracts of *Cassia tora* by Hydrogen Peroxide method

Sl.no	% Inhibition of different extracts						
Concentration (µg/ml)	Petroleum extract	Ether	Chloroform Extract	Ethylacetate Extract	Methanol Extract	Aqueous Extract	Std Ascorbic acid
50	15.49		16.44	14.10	13.75	14.14	14.09
100	38.87		42.95	34.71	34.49	34.16	33.92
150	54.36		62.41	54.93	56.17	48.53	54.27
200	89.01		75.83	83.20	84.61	79.02	78.28
250	100		100	100	100	100	100

Table 8: Showing IC₅₀ Values of different extracts of *Cassia tora*.

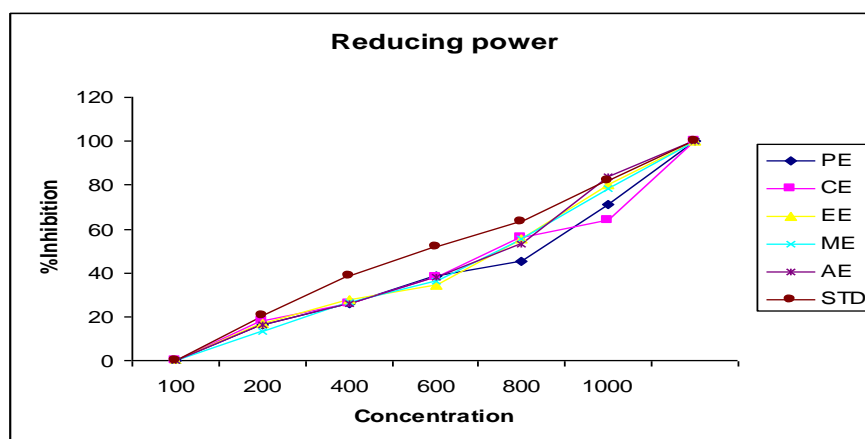
Sl.no	PARTICULARS	IC ₅₀ VALUES(µg/ml)
1	Ascorbic acid	129.97
2	Petroleum ether extract	123.60
3	Chloroform extract	126.16
4	Ethyl acetate extract	127.19
5	Methanol extract	126.19
6	Aqueous extract	131.68

The absorbance of Petroleum ether, Chloroform, Ethyl acetate, Methanol and Aqueous extracts of *Cassia tora* was found to be 0.820, 0.596, 0.893, 0.858, 0.820 respectively and are comparable to standard ascorbic acid whose absorbance was found to be 0.958.

The IC₅₀ values of the Petroleum ether, Chloroform, Ethyl acetate, Methanol and Aqueous extracts of *Cassia tora* was found to be 129.97, 123.60, 121.16, 127.19,

126.17 and are comparable to standard ascorbic acid whose IC₅₀ value was found to be 126.28.

Flavonoids and /or Saponins glycosides can act as free radical scavengers and terminate the radical chain reactions. The antioxidant activity might be due to the presence of flavonoids/ saponins in the leaves of *Cassia tora*[28].


Fig 1: Reducing power of PE, CE, EE, ME and AE of *Cassia tora* at different concentrations.

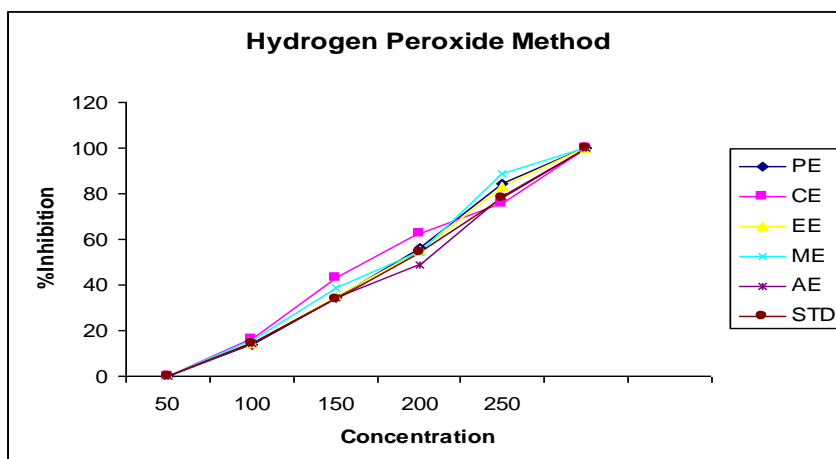


Fig 2: H₂O₂ Scavenging activity of PE, CE, EE, ME and AE of *Cassia tora* at different concentrations.

3. IN VIVO SCREENING OF THE DIFFERENT LEAF EXTRACTS OF *CASSIA TORA* FOR ANTIDIABETIC ACTIVITY USING ALLOXAN INDUCED MODEL

Table 9: Showing blood glucose levels of different extracts of *Cassia tora* before and after treatment

Group	Mean \pm SD blood glucose levels (mg/dl)							
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Control	86.16 \pm 5.1	85.33 \pm 7.2	96.6 \pm 7.5	98.6 \pm 7.4	98.3 \pm 9.7	96.6 \pm 8.3	97.0 \pm 6.0	97.1 \pm 6.9
Diabetic control	255.8 \pm 42.2	250.6 \pm 43.5	253.6 \pm 46.5	250.5 \pm 46.5	250.1 \pm 48.4	251.0 \pm 46.8	251.3 \pm 48	250.6 \pm 49.5
Standard (glibenclamide)	342.3 \pm 20.7	130.8 \pm 15.4**	94.6 \pm 7.5***	96.6 \pm 7.4***	96.3 \pm 9.7***	94.6 \pm 8.3	95.7 \pm 6.03	96.9 \pm 6.9
(400mg/kg) Methanol extract	315.6 \pm 45.3	239.6 \pm 26.2	217.8 \pm 34.5	213.8 \pm 47.0**	181.1 \pm 41.6***	213.6 \pm 42.7	215.5 \pm 49.1	220.5 \pm 50.0
(400mg/kg) Ethyl acetate extract	342.3 \pm 20.7	144.6 \pm 17.9	132.5 \pm 20.1	128.8 \pm 21.4	109.3 \pm 17.1***	123.8 \pm 30.5	127.3 \pm 18.9	132.0 \pm 16.7
(400mg/kg) Aqueous extract	254.5 \pm 31.9	129.1 \pm 19.9	128.5 \pm 21.4	129.5 \pm 12.5	133.3 \pm 20.8***	143.1 \pm 20.4	147.5 \pm 21.9	149.5 \pm 18.4
(200mg/kg) Methanol extract	303.8 \pm 48.8	232.6 \pm 42.8**	216.6 \pm 42.6	203.1 \pm 42.7	164.5 \pm 33.0***	210.3 \pm 40.6	226.3 \pm 39.1	231.8 \pm 39.3
(200mg/kg) Ethyl acetate extract	325.8 \pm 36.4	194.1 \pm 26.7	179.6 \pm 21.2***	184.8 \pm 27.7***	189.0 \pm 22.7	191.8 \pm 22.7	196.6 \pm 22.7	201.0 \pm 21.1
(200mg/kg) Aqueous extract	318.6 \pm 22.4	242.3 \pm 33.5	229.6 \pm 27.2	216.8 \pm 32.7	193.5 \pm 36.7***	221.8 \pm 28.7	222.6 \pm 36.4	226.0 \pm 23.9

***Statistically significant $p < 0.001$, ** $p < 0.01$ compared to 0 hr of their respective group.

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the students t-test.

Table 10: Showing percentage reduction of blood glucose levels with different extracts of *Cassia tora*

Group	Percentage of reduction of blood glucose						
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Control	2.72	2.41	0.50	0.80	2.52	2.11	2.01
Diabetic control	2.03	0.86	2.07	2.22	1.87	1.75	2.03
Standard (glibenclamide)	61.4	72.0	71.4	72.12	72.02	71.9	71.87
400mg/kg. Methanol extract	22.34	30.01	31.2	41.30	31.32	30.7	29.20
400mg/kg Ethyl acetate extract	57.83	61.0	62.1	67.76	62.98	62.55	60.90
400mg/kg Aqueous extract	48.32	48.55	48.1	43.62	41.77	40.46	39.6
200mg/kg Methanol extract	23.43	28.70	33.1	45.8	30.77	25.51	23.69
200mg/kg Ethyl acetate extract	40.4	44.87	43.2	41.98	41.12	39.65	38.30
200mg/kg Aqueous extract	23.6	27.9	32.71	39.30	30.50	29.87	28.30

Alloxan, a beta cytotoxin, includes diabetes in a wide variety of animals by damaging the insulin secreting beta cell resulting in a decrease in endogenous insulin release, which decreases the utilization of glucose by the tissues.

All the three extracts shown antidiabetic activity, but EE at the dose 400mg/kg were found to possess more glucose tolerance activity than 200mg/kg, whereas ME and AE at dose of 200mg/kg possess more glucose tolerance activity than 400mg/kg. (i.e., dose dependent activity was observed with the EE and not observed with ME and AE).

Yen et al., suggested that emodin and obtusifolin in *Cassia tora* might be the components having antidiabetic function since they exhibited inhibitory activity on advanced glycation end products [29]. The significant antidiabetic activity of leaves of *Cassia tora* may be due to inhibition of free radical generation and subsequent tissue damage induced by alloxan or potentiation of plasma insulin effect by increase either pancreatic secretion of insulin from existing beta cells or its release from as indicated by significant improvement in glucose and protein level because insulin inhibit gluconeogenesis from proteins.

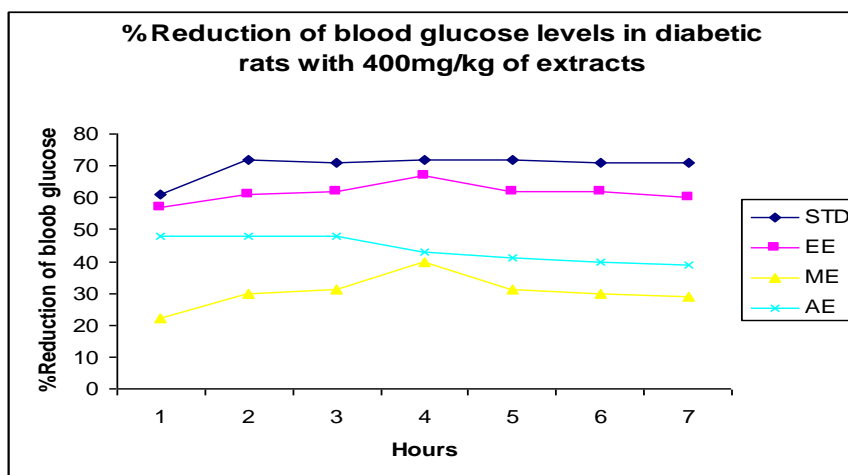


Fig 3: Showing the reduction of blood glucose levels with a dose of 400mg/kg of EE, ME and Aqueous Extracts

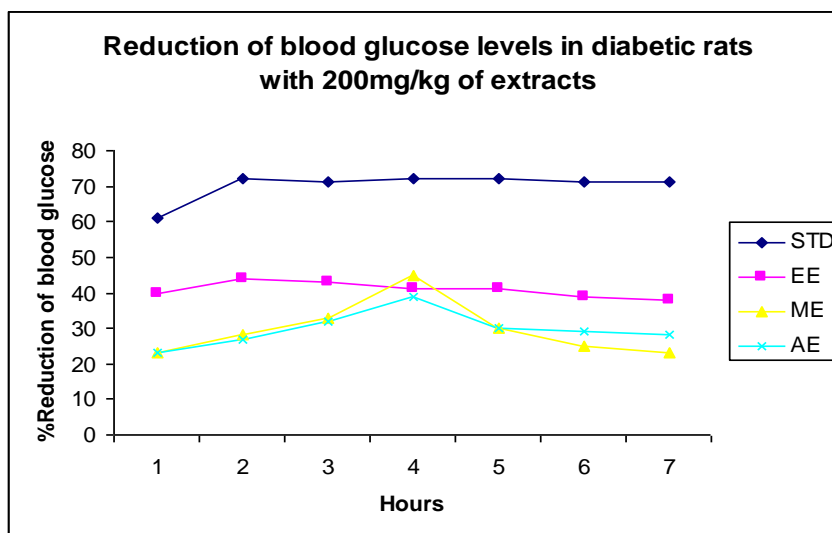


Fig 4: Showing the reduction of blood glucose levels with a dose of 200mg/kg of EE, ME and Aqueous Extracts.

4. EFFECT ON ORAL GLUCOSE TOLERANCE TEST:

After overnight fasting, a zero-min blood sample (0.2ml) was taken from the rats in normal, diabetic control, normal and plant extract treated group,

Glibenclamide treated groups by orbital sinus puncture. Glucose solution (2.5g/kg) was administered orally immediately. Four more samples were taken at 30, 60, 90, and 120min after glucose administration.

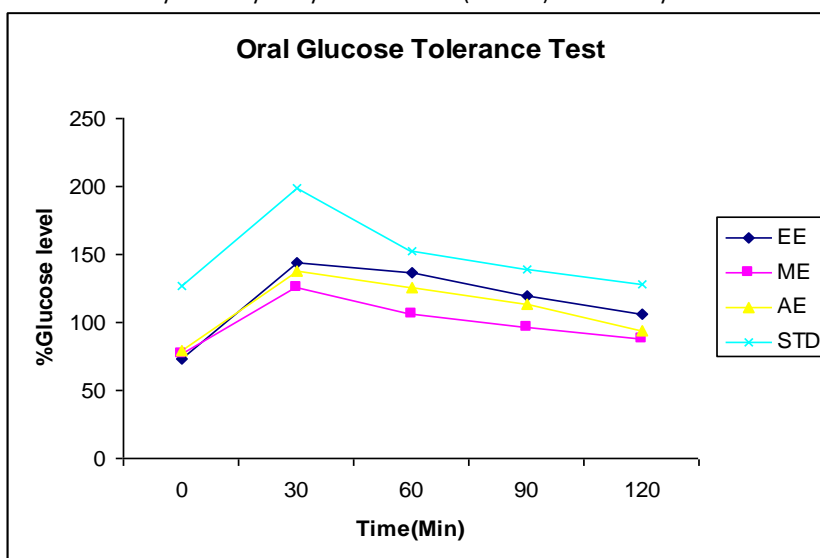
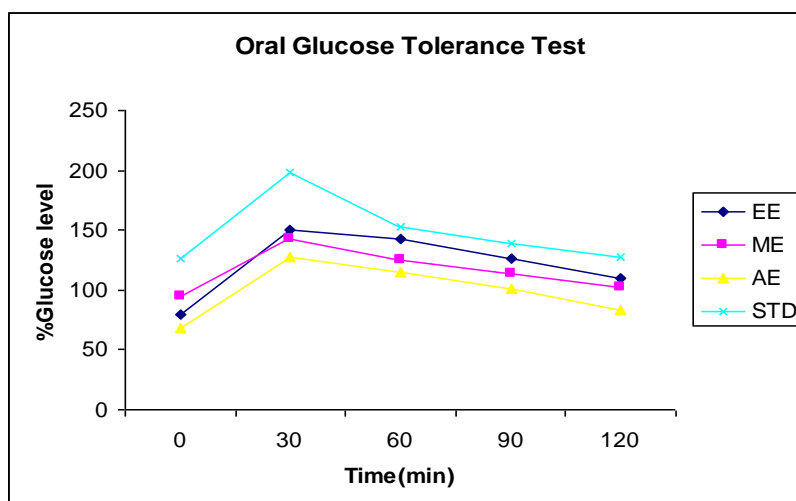
Table11: Showing the blood glucose levels of different extracts of *Cassia tora* in glucose tolerance test

Group	Mean \pm SD blood glucose levels (mg/dl)				
	0 min (Initial) ^a	30min	60min	90min	120min
Control	94.3 \pm 1.8	187.4 \pm 6.1	168.7 \pm 5.2	138.4 \pm 2.7	107.8 \pm 1.8
Diabetic control	258.4 \pm 3.8	338.6 \pm 3.7	387.5 \pm 4.3	364.4 \pm 8.5	333.3 \pm 3.9
Standard (glibenclamide)	126.3 \pm 8.1	198.3 \pm 3.8	156.2\pm3.6***	139.4\pm4.9***	128.3\pm1.5**
(400mg/kg) Ethylacetate extract	73.6 \pm 6.5	144.8 \pm 8.4	137.7 \pm 6.3	120.2\pm8.0**	106.4\pm7.4*
(400mg/kg) Methanol extract	77.3 \pm 2.3	126.7 \pm 6.3	106.5\pm2.2***	96.7\pm3.6**	88.2\pm3.5*
(400mg/kg) Aqueous extract	79.7 \pm 4.3	138.6 \pm 3.7	126.3 \pm 3.4	113.6\pm3.1**	94.3\pm2.1*
(200mg/kg) Ethyl acetate extract	80.9 \pm 6.8	150.3 \pm 3.8	143.3 \pm 1.6	126.2\pm6.3**	110.6\pm2.3*
(200mg/kg) Methanol extract	95.6 \pm 2.3	143.2 \pm 9.3	125.3\pm5.6***	114.2\pm3.6**	102.1\pm3.4
(200mg/kg) Aqueous extract	68.6 \pm 3.9	127.5 \pm 2.3	115.2 \pm 6.4	101.8\pm2.6*	83.6\pm3.3**

***=P<0.001, **=P<0.01, *=P<0.05, compared with 0min.

a. The rats of all groups were loaded with glucose (2.5/kg, P.O) 30min after the administration of different extracts of *Cassia tora*.

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the students *t*-test.


Fig 5: Showing the results of glucose tolerance test with the 400mg/kg of extracts.

Fig 6: Showing the results of glucose tolerance test with the 200mg/kg of extracts.

In glucose-loaded animals, it is possible that the extract may act by Potentiation of the pancreatic secretion or by increasing glucose uptake.

All the three extracts shown increased glucose tolerance, but EE at the dose 400mg/kg were found to possess more glucose tolerance activity compare to that of Glibenclamide, the order of glucose tolerance is EE>ME>AE.

This glucose tolerance activity might be due to the presence of flavonoid glycosides in the *Cassia tora* leaves. However, chemical and pharmacological investigations are necessary to identify the latter and to confirm its mechanism of action and its antidiabetic potential.

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