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Research Article

Evaluation of Antidiabetic Effect of Ethanolic Extract of *Corchorus olitorious* on Alloxan Induced Diabetes in Male Wistar Rats

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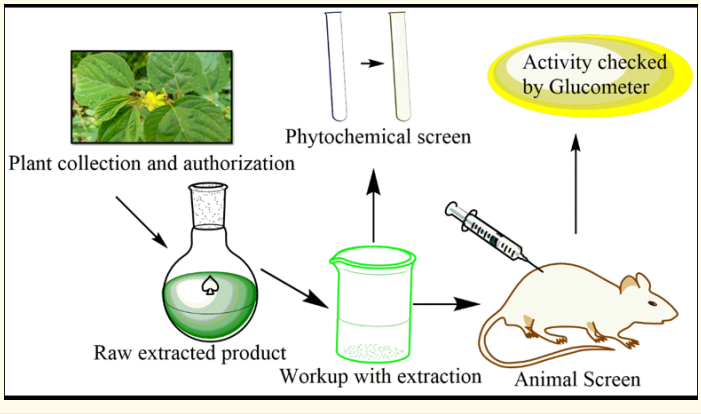
Abstract

Antidiabetic activity of leaf extract of Corchorus olitoriouson Alloxan induced diabetic rats. Diabetes was induced in Male Albino Wistar rats by intraperitoneal injection of aqueous alloxan. The dose was set to 120 mg/kg body weight. Alloxan was induced in one time. Leaves of Corchorus olitorious were collected, dried and powdered with mixer grinder. Coarse powder was taken for the cold maceration extraction process where the solvent was used as petroleum ether and kept for 24 hours. After that dried extract was again extracted with cold maceration techniques where methanol is used as solvent. Excess solvent was separated with simple distillation method. Animals were divided into 4 groups. Normal control group was treated with saline, positive control group was treated with saline and alloxan, test group was treated with alloxan and plant extract (600 mg/kg p.o.) and standard group was treated with alloxan and Glimepiride (5 mg/kg p.o.). Treatment was continued for 10 days. Blood glucose level was measured at 0th, 3rd, 7th and 10th day. The present study showed that the blood glucose level was reduced in test group where it was compared with the positive control group. It showed the antidiabetic activity.

Keywords: *Corchorus olitorious*; Alloxan; Petroleum Ether; Methanol; Glimepiride; Antidiabetic Activity



Introduction

Diabetes is a disease in which the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood. Three types of diabetes are there: Type 1- A chronic condition in which the pancreas produces little or no insulin, Type 2- A chronic condition that affects the way the body processes blood sugar, Gestational diabetes – This is due to insulin-blocking hormones produced during pregnancy [1,2].

According to the IDF (International Diabetes Federation) report, China, India, United States are in top of the list for the most cases of Diabetes [3,4]. Around 463 million adults (20-79 years) worldwide were living with Diabetes. The total number of diabetes patients is expected to be more than double in 2025 [5,6].

Alloxan which is chemically known as 5,5-dihydroxyl pyrimidine- 2,4,6-trione is an organic compound, a urea derivative, a carcinogen and cytotoxic glucose analogue [7,8]. The compound has the molecular formulae, $C_4H_2N_2O_4$ and a relative molecular mass of Alloxan is used to induce diabetes in mice.[9]White Jute (Corchorus olitorious) family Tiliaceae. It is used to treat a variety of chronic and infectious diseases including Diabetes [10,11].

Corchorus olitorious (Tiliaceae) is a climbing perennial herb, growing throughout India and it is widely used in the traditional treatment of diabetes [12]. The aim of present study was to evaluate the antidiabetic potential of the mature unripe of *Corchorus olitorious* in Alloxan induced diabetic rats with special reference to carbohydrate metabolizing enzymes [13]. The optimum dose of *Corchorus olitorious* extract (GCE) was determined by oral glucose tolerance test. The effects of CGE were compared with Glibenclamide [14].

Pathophysiology of Type 1 diabetes

The majority of type 1 diabetes is of the immune mediated variety, where beta cell loss is a T- cell mediated autoimmune attack. [15]T lymphocytes are a part of the immune system [16]. They are type of white blood cell [17]. The CD+ class of the T lymphocytes causes destruction to the pancreatic beta cells. This class of T lymphocytes recognizes the beta cell surface antigens as non-self and trigger apoptotic cell death [18]. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults but was traditionally termed as juvenile diabetes because it represents a majority of the diabetes cases in children [19,20].

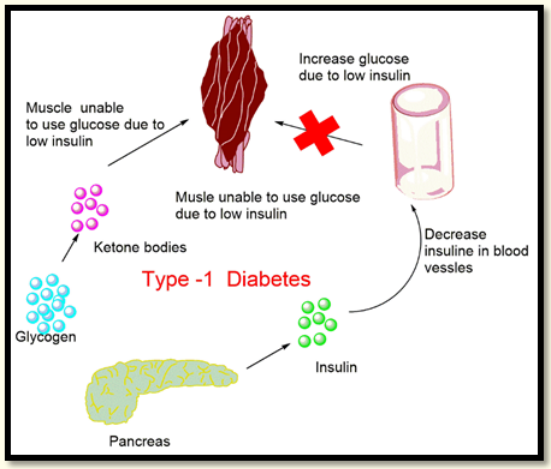


Figure 1: Pathophysiology of Type 1 Diabetes.

Pathophysiology of type 2 diabetes

Type 2 diabetes is also called maturity onset diabetes mellitus. There is no loss or moderate reduction in the β cell mass, insulin in circulation levels is low and generally has a late onset of disease after middle age [21]. This may be due to an abnormality in the glucoreceptors of β cells, therefore, they respond at higher glucose concentrations or at relative β cell deficiency [22]. The reduced sensitivity of peripheral tissues to insulin and reduction in the number of insulin receptors are a consequence for producing diabetes [23]. When glucagon exceeds a normal amount, it produces hypoglycemia [24].

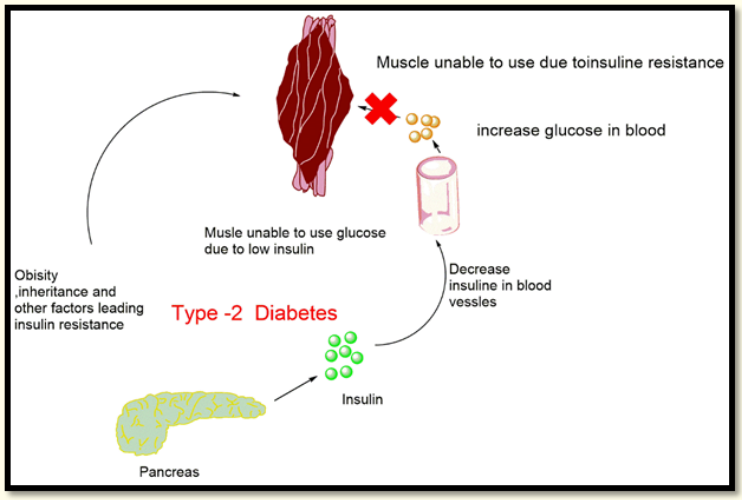


Figure 2: Pathophysiology of Type 2 Diabetes.

Pathophysiology of gestational diabetes

This type of diabetes occurs in women during pregnancy when the body can become less sensitive to insulin. Gestational diabetes causes high blood sugar that can affect pregnancy and baby's health [25]. In women with gestational diabetes, blood sugar usually returns to normal soon after delivery. But if someone had gestational diabetes, there is a high risk of getting type 2 diabetes [26-29].

Materials and Methods

Selection of plant

The plant *Corchorus olitorious* was selected for this good medicinal value. It has been used in traditional medicine as household remedy for various diseases. The whole part of *Corchorus olitorious* having pharmacological activities like analgesic, antipyretic, anti-inflammatory, antimicrobial, antioxidant, hypoglycemic, antimalarial, demulcent, diuretics. It is easily available in market as well as well as in rural areas. It is generally cultivated on wide range of loamy soil with moderate fertility. Plant contains Vitamin C, beta carotene, glutathione, phenols. The whole plant is traditionally used for various medicinal purposes. leaves of the plant are used in Indian folk medicine for treatment of number of ailments including diabetes, wounds, ulcers, inflammation, in eruptions of skin, fever, asthma, and cough.

Collection and authentication of plant

The studies have been carried out on the anti-diabetic properties of ethanolic extract of *Corchorus olitorious* leaves, sample was collected from the rural belt of Purba Medinipur, West Bengal, India in September 2022. The plant was separated from undesirable materials, cleaned, washed with distilled water, and shade dried at room temperature. After that, the plant was authenticated bythe Zoological Survey of India, Botanical Garden, Howrah.

Extraction process

The shade-dried leaves were powdered using a mechanical grinder. Powder (500 gm) was first extracted with 450mL of petroleum ether for 24 hours, then with 500 mL of ethanol for 72 hours through the maceration process. The solvents used were of analyti-

cal grade. Ethanol was removed from the extract under vacuum and a semisolid mass was obtained. It was stored in sterile amber-colored storage vials in the refrigerator until used for the experiment.

Study of phytochemical screening [30-33]

Test of carbohydrates

- **Molisch's Test:** To 1 mL extract solution added 1 mL of α-napthol and of conc. Sulphuric acid from the wall of the test tube.
- **Fehling's Test:** To the extract solution Fehling- A and Fehling-B reagents was added drop by drop. Then the solution was heated on boiling water bath.
- **Benedict Test:** To the extract solution Benedict's reagent was added. The solution was mixed well and allowed to heat on a boiling water bath for 5 minutes.

Test for alkaloids

- **Mayer's Test:** To few mL of extract solution mercuric and potassium iodine were added.
- **Wagner's Test:** To 1 mL of extract solution potassium few drops of iodine and potassium iodide were added.
- **Dragendroff's Test:** To 1 mL of extract solution potassium bismuth nitrate was added.
- **Hager's Test:** To 1 mL of extract solution few drops of picric acid was added.

Test of tannins

1 gm of plant grinded, ten samples was boiled in 20mL 70% ethanol for 2 min. The mixture was filtered and a portion of the filtrate diluted with water in a ratio of 1:4 and 3 drops of 10% ferric chloride solution added. The resulting solution was bluish black.

The extract treated with potassium dichromate solution.

- **Lead acetate Test:** To 2-3 mL of the test solution lead acetate solution was added.

Test of steroids

- **Liberman Buchard's Test:** 200 mg plant material boiled in 10 mL of chloroform and the mixture was filtered; 2 mL of filtrate was added to 2 mL acetic anhydride and conc. Sulphuric acid.
- **Salkowski Test:** To 2 mL of test solution few drops of cons sulphuric acid was added, shaken and allowed to stand for sometimes.

Test of glycosides

- **Keller Killiani test:** A solution of 0.5mL containing glacial acetic acid and 2-3 drops of ferric chloride was mixed with 2mL of extract. Later, 1mL of conc H_2SO_4, was added along the walls of test tube. The appearance of deep blue colour at the junction of liquids indicates the presence of cardiac glycoside.
- **Borntrager's test:** To 1 gm of drug add 5–10 mL of dilute HCl boil on water bath for 10 min and filter. Filtrate was extracted with CCl4/ benzene and add equal amount of ammonia solution to fil trate and shake. Formation of pink or red colour in ammoni-cal layer due to presence of anthraquinone moiety.
- **Modified Borntrager's test:** To 1 gm of drug, add 5 mL dilute HCl followed by 5 mL ferric Chloride (5% w/v). Boil for 10 min on water bath, cool and filter, filtrate was extracted with carbon tetra- chloride or benzene and add equal volume of ammonia solution, formation of pink to red colour due to presence of anthraquinone moiety. This is used C-type of anthraquinone glycosides.

Tests for saponins

A drop of Na_2CO_3 solution was added to 5 mL of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponins.

Experimental animals

Male Albino Wistar Rats (125-150gm), maintained under controlled conditions of temperature (23 ± 2°c), humidity (50 ± 5%) and a 12h light-dark cycle, were used for the experiment. They were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard rat pellet diet and water ad libitum. The animals were given a week's time to get acclimatized with the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA), ministry of social justice and empowerment Government of India, norms and approved by the Institutional Animals Ethics Committee (IAEC). The animals were anesthetized by treated with propofol forcollecting the blood samples from the orbital venous. Propofolwas administered intraperitoneally [34,35].

Acute oral toxicity

The acute oral toxicity study was conducted according to Organization for Economic and Cultural Development for testing of chemicals (OECD, 2001, 423 guideline) and up and down method was used for the study. 3 male rats were taken for toxicity testing. The wistar rats were weighed (125-150 gm), and were treated with oral dose i.e. (1st dose- 300 mg/kg, 2nd dose- 2000 mg/kg) of ethanolic extract of *Corchorus olitorious* roots respectively for 2 different days.

- Firstly, 300 mg/kg drug was administered, after the administration of the 1st dose of the A *Corchorus olitorious* root extract and food was withheld for a further 3-4 hrs. The animals were observed individually at least once during the first 30 min after dosing, periodically at 8, 14, 24, and 48 hrs intervals.
- After passing of the 1st dose and observing the survival of the rats, 2nd dose 2000 mg/kg of the *Corchorus olitorious* root extract was administered and food was withheld for a further 3-4 hrs. The animals were observed individually at least once during the first 30 min after dosing, periodically at 8, 14, 24, and 48 hrs intervals.
- Since 2000 mg/kg drug seems to be passed on the rats, therefore this dose is considered as the safe dose (LD_{50}).

The animals were observed for signs of drowsiness, hair loss, loss of appetite, salivation, tremors, convulsion and bulging of the eyes. The animals were thereafter observed for a period of 14 days for any signs of delayed toxicity and mortality.

Housing of animal

- The Albino Wistar rats were collected and transported in the plastic ventilated cages and had free access to standard rat pellet diet and water ad libitum.
- Albino Wistar rat (male)weighing 125-150g, were maintained under the controlled conditions of temperature (23 ± 2°c), humidity (50 ± 5%) and a 12h light-dark cycle, will be used for the experiment.
- The rats were given necessary food and water for nutrition.
- Identification of rats were done in head, body, tail, legs by using the picric acid and then were separated in 4 groups.

Grouping of animals

Animals were divided into 4 groups. Negative Control treated with normal saline. Positive control treated with Alloxan. Whereas test group treated with plant extract and inducing agent and standard group treated with Glimepiride and inducing agent [36]. It was depicted in Table 1.

Table 1: Grouping of Animals.

Group1	Group2	Group3	Group4
Negative Control	**Positive Control**	**Standard Group**	**Test Group**
Six rats were treated with Normal Saline water	Rats were treated with Alloxan	Rats were treated with Glimepiride. (5mg/kgp.o)	The rats were treated with ethanolic leaf extract of *Corchorus olitorious* (600 mg/kgbody weight)weight) for5 successive days.

Statistical analysis

Result was expressed Mean ± SEM from six animals in each group. Comparison between the groups will be made by using one way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test using GraphPad prism version 4, where $p < 0.05$ was considered as statistically significant.

Results

Morphological identification

Macroscopical Character: Macroscopical identification of this plant is given below.

Morphological Characters	*Corchorus olitorious*
Leaf arrangement	Alternate distichous
Leaf type	Simple
Leaf Shape	Broad lanceolate
Leaf apex	Obtuse-mucronate
Leaf base	Subrotund-cordate
Leaf margin	Lobed
Flower	Solitary
Fruit	Dehiscent capsule, fusiform.

Table 2: Macroscopical Character.

Microscopical character

T.S. of root shows

In Transverse section of root, epidermis consists of closely packed, elongated cells with single layer. Cuticle is present on the epidermis. Unicellular epidermal hairs present. Cortex is made up of parenchymatous cells. The cells are polygonal in shape. Cortex is thin walled, massive and inter-cellular spaces. Endodermis uniseriate, pericyclic, is of thin-walled parenchyma. Xylem and phloem are alternately arranged in the vascular bundle, cambium is absent and pith is scanty at the centre.

T.S. of stem shows

This plant is a perennial climber with single tendrils and glabrous leaves. The leaves have 5 lobes and are 6.5–8.5 cm long and 7–8 cm wide. Female and male flowers emerge at the axils on the petiole, and have 3 stamens.

Ridged Sponge Gourd is a tropical running vine with rounded leaves and yellow flowers. Both female and male flowers appear on the same plant. Pollination is done by bees. Leaves are angled, but little if at all lobed except on young shoots. The leaves are covered with short hairs and the fruits are ribbed and cylindrical shaped.

T.S. of leaf passing through mid-rib shows

In Transverse section of root, epidermis consists of closely packed, elongated cells with single layer. Cuticle is present on the epidermis. Unicellular epidermal hairs are present. Cortex is made up of parenchymatous cells. The cells are polygonal in shape. Cortex is thin walled, massive and inter-cellular space Endodermis uniseriate, pericyclic, is of thin-walled parenchyma. Xylem and phloem are alternately arranged in the vascular bundle, cambium is absent and pith is scanty at the center.

Phytochemical screening

Phytochemical constituent	Test	Result
Carbohydrate:	Molisch test	+
	Fehling's test	+
	Benedict's test	+
Alkaloids:	Mayer's test	+
	Wagner's test	+
	Dragendroff's test	+
	Hager's test	+
Tannins:	Ferric chloride	+
	potassium dichromate	+
	Lead acetate solution	+
	Bromine water	_
	Dilute iodine solution	+
Saponin	Foam Test	+
Proteins and Amino acid test		_
Steroids		_

Table 3: Result of phytochemical screening.

(+) indicates presence; (-) indicate absence.

Acute oral toxicity test

In toxicity study, no mortality occurred during 48 hours of observation with the selected dose 2000 mg/kg p.o. As the ethanolic extract of *Corchorus olitorious* leaf were found to be tolerated up to a dose level of 2000 mg/kg p.o. The extract was considered to be safe and dose ranges 500 mg/kg p.o. (1/4th of the extract) and 700 mg/kg p.o. (1/3rd of the extract) was selected for the present study.

Experimental observations

Table 4 depicted that, on 10th day blood glucose level was found to be 87 mg/dL in negative control group. Where, in positive con-

Group	Treatment	0 Days	3 Days	7 Days	10 Days
I (Negative)	Normal Saline	86 ± 1.96	88 ± 2.01	90 ± 1.96	87 ± 1.47
II (Positive Control)	Alloxan (120 mg/kg)i.p.	89 ± 2.83	210 ± 6.37	270 ± 3.40	274 ± 1.78
III (Standard)	Alloxan+ Glimepiride (5 mg/kg p.o)	92±1.86	190 ± 13.26	154 ± 2.31	98 ± 2.90
IV (Test Group)	Alloxan+*Corchorus olitorious* (600 mg/kg p.o)	86 ± 213	182 ± 3.86	110 ± 2.49	81 ± 2.26

Table 4: Overall observation of blood glucose level

N = 6 no of animals. All the values are very significant p<0.05. Significant differences between means were evaluated by Student's t-test

trol group, it was found to be 274 mg/dL. In standard group/, it was found to be 98 mg/dL where in test group (*Corchorus olitorious* extract), it was found to be 81 mg/dL. From the above table, it was found that test group is more significant than the others group. As compared with the others group, the blood glucose level of the test group (*Corchorus olitorious* extract) was reduced and controlled. So, this group is considered as most significant group. It is also depicted through statically which is shown below.

Statistical analysis report

Figure 3: Plant *Chorchorus olitorious.*

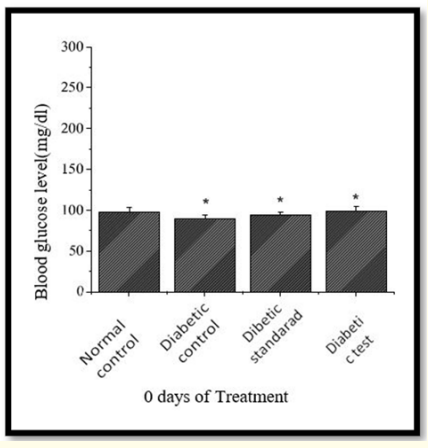


Figure 4: Effect of ethanolic extract of leaves of *Corchorus olitorious* in Alloxan induced rat at 0 day.

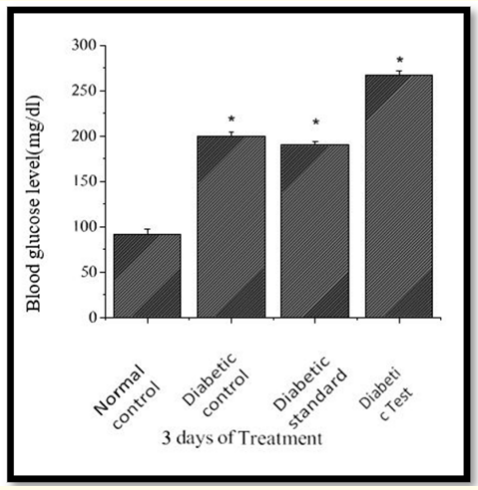


Figure 5: Effect of ethanolic extract of leaves of *Corchorus olitorious* in Alloxan induced rat at 3 days.

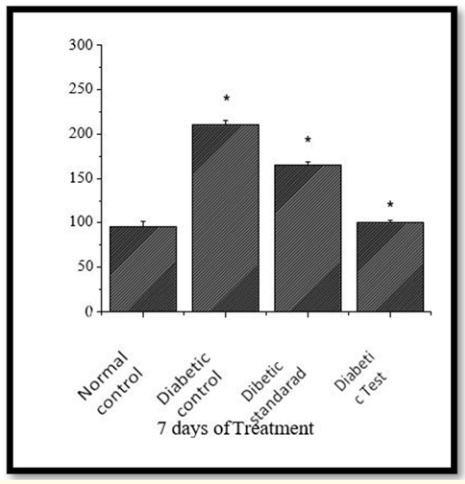


Figure 6: Effect of ethanolic extract of leaves of *Corchorus olitorious* in Alloxan induced rat at 7 days.

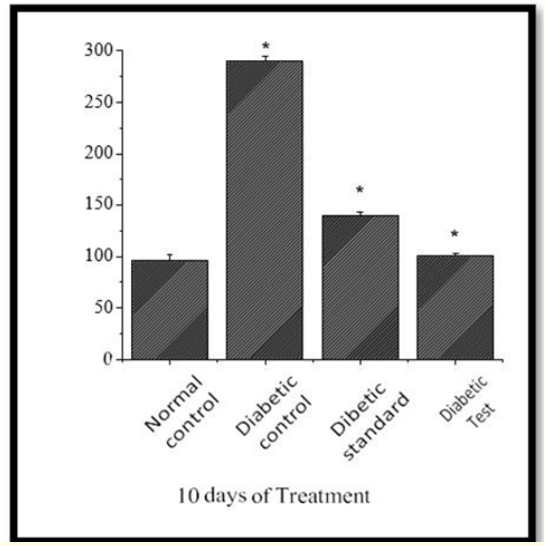


Figure 7: Effect of ethanolic extract of leaves of *Corchorus olitorious* in Alloxan induced rat at 10 days.

Discussion

Practically all the plants and their parts were discovered to be wealthy in phytochemicals, for example, terpenes, terpenoid. Terpenes are the most widely recognized terpenoids [37,38]. Terpenoids concentrate of *Corchorus olitorious* has a great anti-diabetic property, which is effectively seen in this examination. Other hand in this investigation we examine about the number of cycles were included a medication advancement, how might we direct a preclinical preliminary as well as clinical preliminaries. How might we have isolated, separated and recognized the medication particle, readiness a dose structure and so on and portion estimation by LD_{50} (intense harmfulness test) [39].

Alloxan which is chemically known as 5,5-dihydroxyl pyrimidine-2,4,6-trione is an organic compound, a urea derivative, a carcinogen and cytotoxic glucose analogue [40]. The compound has the molecular formulae, $C_4H_2N_2O_4$ and a relative molecular mass of Alloxan is used to induce diabetes in mice [41]. The diabetogenic action of Alloxan is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin [42].

Carcinogenesis, teratogenesis and mutagenesis, it is diabetogenic, hepatotoxic, nephrotoxic and also causes gastric ulceration [43]. Alloxan given intravenously, intraperitoneally to laboratory rat in multiple sub- diabetogenic doses induces pronounced pancreatic insulitis with eventual destruction of insulin secreting beta cells and diabetes mellitus. The incidence and severity of lesion produced by Alloxan in pancreas, liver, kidney and GIT, progressively increased with the time post treatment [44].

Alloxan has two distinct pathological effects: it selectively inhibits glucose- induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin- dependent diabetes through its ability to induce ROS formation, resulting in the selective necrosis [45,46].

The ethanolic extract of leaves of *Corchorus olitorious* decreases glucose level in Alloxan induced diabetic in rat. Hence, *Corchorus olitorious* extraction may effective in some pathway which aggravate the diabetes mellitus. Such this experiment indicates this *Corchorus olitorious* extraction may stimulate the secretion of insulin or the activity of insulin increase [41-43].

Conclusion

One of the most prevalent global health issues, diabetes mellitus has a sharply rising incidence. Since the dawn of time, a range of medicinal plants, particularly those from the family Tiliaceae and the genus Corchorus, have been used to treat chronic diseases like diabetes. This study showed that the ethanolic extract of *Corchorus olitorius* leaves significantly reduced blood sugar levels. This may support the use of the *Corchorus olitorious* species in etiopathic diabetes mellitus treatment.

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Conflict of Interest

Authors declare that they have no conflict of interest.

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