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PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS OF LEAVES OF *CORCHORUS TRILOCULARIS*

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ABSTRACT

Diabetes has been recognized as a major health problem worldwide for the twenty-first century. Developing countries of Asia and Africa are the most viable areas where the disease is feared to raise 2–3 folds. A survey revealed that it is expected to increase to 366 million people by 2030 worldwide In particular, the number of people with diabetes in India is around 40.9 million by 2004 and is expected to rise to 69.9 million by 2030. India leads the world with largest number of diabetic subjects thus earning the dubious distinction of being termed the ‘‘Diabetes Capital of the World”. The disease imposes huge human and economic costs on patients, their families, local communities, health care systems, and societies. Diabetes mellitus was known to ancient Indian physicians as ‘madumeha’. Diabetes mellitus (DM) is a chronic and metabolic disease affecting glucose, fat, and protein metabolism Diabetes is defined as a state in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action or both at one or more points in the complex pathways of hormone action. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis doesn’t return to normalcy and continuous for a protracted period of time, it leads to hyperglycemia that is due course turns into a syndrome called diabetes mellitus

KEYWORDS: Diabetes mellitus, insulin secretion, hyperglycemia, carbohydrate.

INTRODUCTION

Diabetes mellitus was known to ancient Indian physicians as ‘madumeha’ Diabetes mellitus (DM) is a chronic and metabolic disease affecting glucose, fat, and protein metabolism Diabetes is defined as a state in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action or both at one or more points in the complex pathways of hormone action. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis doesn’t return to normalcy and continuous for a protracted period of time, it leads to hyperglycemia that is due course turns into a syndrome called diabetes mellitus DM causes complications such as nephropathy, neuropathy, retinopathy, blindness, obesity, limb amputation and failure of various organs, in particular the blood vessels and nerves and increases mortality rate.

PLANT PROFILE

The genus Corchorus contains an estimated 40 to 100 species of flowering plants native to tropical and subtropical regions throughout the world[1] (The crop ‘jute’ belongs to the genus Corchorus and is the most important natural fibre crop next to cotton.[2] Jute is a native plant of tropical Africa and Asia but also has been spread to Australia, South America and some parts of Europe. It has been grown extensively in India, Bangladesh, China, Myanmar and Nepal.

Chemical Constituents: Penta cyclic triterpenoids betulinic Acid and steroid β-sitosterol-D-glucoside have been isolated, on chemical examination it was concluded that, flavonoids were present in whole plant extract. cardiac glycosides strophanthidin (1) -3-ß-D-bolvinosido -ß-D-glucoside corchoroside A, components of glycoside mixture – olitoriside. Triterpenoids such as, Oxocorosin**,** Urosolic acid and corosolic acid have been isolated from Corchorus *trilocularis.*[3] Plant seeds of Corchorus capsularis comprised of corchorin, corchortoxin helveticoside, cardiac glycosides, corchoroside A and B,

olitoriside, erysimoside, strophantidol glycosides, biosides, oliogosaccaride and olitoriside; whilst leaves comprised of saponins, flavonoids, glucoside, capsularin steroids triterpenes and several various secondary metabolites. The pharmacological experiments unveiled the fact that the plant possessed anti-inflammatory, analgesic, antipyretic, cardiac, antioxidant, antimicrobial, insecticidal and several additional pharmacological properties.[4]

Traditional Uses: The leaves are tasty and sourly, cooling laxative, stimulant, tonic, and aphrodisiac; destroy "tridosha". The edible leaves of Corchorus species are reported to contain some trace minerals useful to alleviate mineral deficiencies of the human body. The seeds are used to removes tumors, pain, stomach troubles, skin diseases, and scabies. The leaves are reported to pr event cardiovascular disorders.[5]

The leaves from Corchorus capsularis have been reported to hold demulcent, laxative, appetizer, stimulant and stomachic and its infusion is customarily used to cure constipation, dysentery, fevers, liver problems as well as dyspepsia. Additionally, decoction of the roots, as well as unripe fruits, has long been used to combat dysentery. The leaves of capsularis are actually consumed as vegetables in numerous area of the world such as Bangladesh, Africa, Middle East and Southeast Parts of Asia, which include Malaysia, for a long period.[6]

MATERIALS AND METHODS

Collection and authentication of the plant leaves: The leaves of *Corchorus trilocularis* were collected from Vindhy herbal botanical garden, Bhopal, during the month of Oct that shows the green color with rough surface. The plant leaves were washed thoroughly in tap water, dried in shade, finely powdered and used for successive extraction methods. Plant was identified by Department of Botany, Safia College of Science and Education Bhopal (MP) India.

Determination of Physico chemical parameters

Ash values are helpful in determining the quality and purity of crude drug, especially in the powder form. The ash content of crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. The total ash of a crude drug reflects the care taken in its preparation. The acid insoluble ash is a part of the total ash which is insoluble in dilute hydrochloric acid. A higher limit of acid- insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. The total ash usually consists of carbonates, phosphates, silicates and silica. Sulphate present in the drug on long storage gets converted in to carbonates and oxide. On treatment of drug with conc. H_2SO_4 the carbonates and oxides get reconverted to sulphate which is stable at high temperature.

Determination of total Ash value[7]

Accurately weight about 3 gm of air dried drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug. The determination of total ash value was calculated by using following formula.

Determination of acid insoluble Ash value: The ash obtained as directed under total ash was boiled with 25 ml of HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Determination of water-soluble Ash value: The total ash obtain was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water ignited for 15 minutes. The weight of insoluble matter was subtracted from the weight of total ash. The differences in weight represent the water soluble ash. The percentage of water-soluble ash was calculated with reference to the air dried drug.

Determination of Extractive values: Extractive values of crude drug are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Determination of Alcohol Soluble Extractive Value The 5 gm. of air dried coarse powder of crude drug was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filter rapidly taking precaution against loss of solvent. Out of that filtrate, 25 ml of filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105^0c and weighed. The percentage of ethanol soluble extractive value was calculated with reference to air dried drug. Determination of Water-Soluble Extractive Value: The 5 gm. of air dried coarse powder of crude drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filter rapidly taking precaution against loss of solvent. Out of that filtrate, 25 ml of filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105^0c and weighed. The percentage of water-soluble extractive value was calculated with reference to air dried drug.

Determination of Loss on Drying: Weight about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100^0c or 105^0c. Cool in a desiccators and watch. The loss in weight is recorded as moisture.

Determination of Moisture Content: About 10g of leaves (without preliminary drying) after accurately weighing was placed in a tarred evaporation dish. It was then dried at 105^0C for 5 hours and weighed. Drying was continued and the leaves were weighed at 1 h interval until the difference between two successive weighing corresponded to not more than 0.25 percent. Constant weight was reached when two consecutive weighing after drying for 30min. and cooling for 30min. in a desiccator, did not show more than 0.01g difference.

Extraction methods[8]: The correctly identified plant leaves is dried in shade at room temperature & after 4-5 days, it is formed in powder by mixer grinder. These should be extracted with Petroleum Ether. Powdered drug 100gm was weighed and packed in soxhlet. The drug was continuously extracted with petroleum ether for about 72 hours. Complete defatting was ensured by placing a drop form the thimble on a filter paper give any oily spot. The mare was dried in air to remove traces of petroleum ether.

Chloroform extraction: Defatted drug was subjected to extraction with chloroform in soxhlet apparatus, the extraction was completed in 17-18 hrs. The extract was dried & stored in dark place.

Ethanolic extraction: Drug was subjected to extraction with ethanol (90%) in soxhlet apparatus, the extraction was completed in 25 cycles. The extract was dried & stored in dark place Preparation of Doses: Doses equivalent to 200 mg and 400 mg of the crude drug per kilogram body weight were calculated, and suspended in 1% w/v tween 80 solutions for the experiment. Preparation of Doses: Doses equivalent to 200 mg and 400 mg of the crude drug per kilogram body weight were calculated, and suspended in 1% w/v tween 80 solutions for the experiment.

Streptozotocin (STZ) induced diabetes in rats: After fasting 18 hours, the rats were injected intraperitoneal injection through tail vein with a single dose of 40 mg/kg Streptozocin freshly dissolved in citrate buffer (pH 4.5). After injection, the rats had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock. Diabetes in rats was observed by moderate Polydipsia and marked Polyuria. The diabetes was confirmed by estimating the blood glucose level after 3 days by glucometer based on glucose oxidation method. Rats having blood glucose level more than 250 mg/dl were selected for further study.

Experimental Design: In order to assess the anti-diabetic activity, the animals were divided in Nine groups of six animals in each group.

(I) Group 1: Normal control, 0.9% NaCl-treated animals
(II) Group 2: Diabetic control, STZ -treated rats (40 mg/kg body weight)
(III) Group 3: Treated with Pet. Ether extract of Corchorus trilocularis (200 mg/kg body weight)
(IV) Group 4: Treated with Pet. Ether extract of Corchorus trilocularis (400 mg/kg body weight)
(V) Group 5: Treated with Chloroform extract of Corchorus trilocularis (200 mg/kg body weight)
(VI) Group 6: Treated with Chloroform extract of Corchorus trilocularis (400 mg/kg body weight)
(VII) Group 7: Treated with Ethanolic extract of Corchorus trilocularis (200 mg/kg body weight)
(VIII) Group 8: Treated with Ethanolic extract of Corchorus trilocularis (400 mg/kg body weight)
(IX) Group 9: Treated with Glibernclamide (5 mg/kg body weight

The test drug and reference drug were administered orally at two dose level for a period of 21 days from starting day of diabetes.

Blood collection and biochemical estimations in serum & Pancreas: On 22nd day, fasting blood samples were collected from the tail vein of all the groups of rats. Whole blood was collected for estimation of blood glucose by using the glucometer glycosylated hemoglobin and glutathione Serum was separated for estimation of specific serum marker enzymes, namely, lactate dehydrogenase and creatine kinase . Streptozocin-induced oxidative stress in diabetes is also a predictor of cardiac damage. Since LDH and CK are specific cardiac marker enzymes, increased serum LDH and CK levels were considered as marker of oxidative stress-induced cardiac damage.

Biochemical estimation in pancreatic tissue: After blood collection, all the animals were sacrificed and pancreas was dissected out. Tissue was washed with ice cold saline, weighed and minced; 10% homogenate was prepared in 0.15M ice-cold KCl for TBARS a marker for lipid per oxidation and protein estimation; in 0.02M EDTA for glutathione estimation and in phosphate buffer (pH 7.4) for superoxide dismutase (SOD).

RESULT AND DISCUSSION

Determination of physicochemical parameters.

Table 6.1: Physico-chemical parameters of *Corchorus trilocularis.*

S. No.	Determination	% age
1	Total ash	8.0
2	Acid insoluble ash	3.12
3	Water soluble ash	4.96
4	Alcohol soluble extract value	5.40
5	Water soluble extract value	3.90
6	Loss on Drying	6.98

Determination of Moisture content: The study on selected plant material shows that the difference of two consecutive weighing after drying for 30min. and cooling for 30min. in a desiccators- 0.09 gm.

% Yield Determination and Characteristic views of *Corchorus trilocularis* extracts.

Table 6.2: %Yield (w/w) of extracts *Corchorus Trilocularis.*

S No.	Solvent	Wt. of the extract	%Yield (w/w)
1	Petroleum ether	5.29gm	5.29
2	Chloroform	5.18 gm	5.18
3	Ethanol	5.28gm	5.28

Phytochemical Screening: Phytochemical screening of different extracts showed the presence of different phytochemical.

Table 6.3: Preliminary Phytochemical test for different extracts of leaf of *Corchorus Trilocularis.*

S. No.	Test	Petrol eum ether	Chloroform	Methanol
1.	Carbohydrate Molish test Felling test	- -	- -	+ +
2.	Glycosides Bronteger test	-	-	+
3.	Alkaloid Mayer test Hager test	- -	+ +	+ +
4.	Phytosterol + Triterpinoids Salkowaski test	-	+	+
5.	Protein + Amino acid Biuret test Ninhydrin test	- -	- -	- -
6.	Phenolic test Ferric test Lead acetate test	- -	+ +	+ +
7.	Flavonoids Alkaline test	-	-	+
8.	Saponin Foam test	-	-	+
9	Mucilage Iodine test Ethanol test			

Note: (+) ve indicates positive result, whereas (–) ve indicates negative result.

Streptozotocin induced antidiabetic activity Effect on Blood glucose level

The induction of diabetes with streptozotocin increases the blood glucose level significantly ($p<0.001$) in group II rats as compared to normal rats. In 21 day study glibenclamide the standard drug restored the blood glucose highly significantly with the $p<0.001$ in 14 days whereas chloroform extract (200 & 400 mg/kg) reduced the glucose level moderately and highly significant with $p<0.01$ & $p<0.001$. Petroleum ether, and ethanolic extracts had moderately significant effects ($p<0.01$) on 14th and 21st days. The results are shown in table 6.4and fig 6.2, 6.3 and 6.4.

Table 6.4: Effect of different extracts on glucose level in streptozotocin induced diabetic rats.

Group No	Group	Blood Sugar level				
		Long Term Study (Days)				
		Before inducing Diabetes	3	7	14	21
I	Normal control	80.3 ± 0.46	82.2 ± 0.17	81.4 ± 1.7	81.9 ± 0.57	80.11 ± 0.18
II	Diabetic control	82.4 ±0.81	241.7 ±1.89	274.8 1.43***	267.3 ±3.07 ***	290.1 0.24***
III	Pet. Ether	79.4 ± 0.92	241.6 ±1.44	238.9 ±2.11**	233.8±2.98	225.6±0.20*

	extract (200 mg/kg)				**	*
IV	Pet. Ether extract (400 mg/kg)	83.77±1.08	243.4 ±3.04	222.3± 2.89**	216.8±3.09 **	204.4±0.49* *
V	Chloroform extract (200 mg/kg)	84.27±1.09	244.4± 3.05	218.2±2.89**	204.8±3.0***	199.2±0.29***
VI	Chloroform Extract (400 mg/kg)	87.7± 1.09	245.6± 3.09	210.± 2.79***	195.6±3.02***	179.3±0.82* **
XI	Ethanolic extract (200 mg/kg)	81.4 ± 0.84	243.6± 1.46	223.9 ± 2.19	216.8±2.99 **	214.6±1.80**
XII	Ethanolic extract (400 mg/kg)	79.4 ± 0.22	244.6± 1.39	221.2 ± 2.18	213.8±2.88 **	213.6±3.20**
XV	Glibernclamide (5 mg/kg)	83.25±0.97	244.8± 2.54	199.4± 3.49**	169.3±2.77***	160.8±0.24***

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups,

Effect on Different serum parameters: Table 6.5 and fig 6.9, 6.10, 6.11 and 6.12 Shows the levels of glycosylated hemoglobin and levels of enzymes (glutathione, CK, and LDH). The dose of streptozotocin significantly elevated the level of glycosylated haemoglobin in group II diabetic control rats. After treatment with chloroform extract of *Corchorus trilocularis*, the level of glycosylated hemoglobin was significantly lowered in both doses. The levels of blood glutathione in diabetic rats (group II) were significantly lowered (p< 0.001) when compared with those in normal control rats of group.

I. Treatment with chloroform extract (200 mg/kg and 400 mg/kg) for 21 days significantly restored the blood glutathione (GSH) levels as compared to group II rats. Glibenclamide treatment showed highly significant (p< 0.01) increase in blood GSH levels when compared to group II. Petroleum ether, ethanolic and methanolic extracts had moderately significant effects in treated groups.

Furthermore, the levels of CK and LDH were significantly increased in group 2 diabetic rats. However, the test drug treatment for 21 days significantly reduced the levels of CK (p< 0.01 with 200 mg/kg and 400 mg/kg) when compared to diabetic rats. The administration of chloroform, petroleum ether and ethanolic extract moderately significantly reduced (p<0.01) the serum LDH levels in both doses when compared to pathogenic diabetic control rats and the results were comparable to glibenclamide treatment.

Table 6.5: Effect of different extracts on glycosylated haemoglobin, blood glutathione, serum creatine kinase, serum lactate dehydrogenase.

Gro up No	Group	Whole blood HbA1C (%)	Blood GSH (mg/dL)	Serum Creatinine Kinase (CK), (IU/L)	rum LDH (IU/L)
I	Normal Control	5.12 ± 0.22	3.19 ± 0.15	69.14 ± 2.88	190.12± 5.40
II	Diabetic control	15.33±0.42**	1.10 ± 0.10*	153.32 ± 3.91**	311.44±8.11***
III	Pet. Ether extract (200 mg/kg)	13.58 ± 0.24	1.82 ± 0.32*	145.48 ± 1.90*	298.71±10.67*
IV	Pet. Ether extract (400 mg/kg)	12.34± 0.11*	1.85 ± 0.22*	140.28 ± 0.75**	272.21±9.40**
V	Chloroform extract (200 mg/kg)	9.22± 0.14**	1.97 ± 0.21**	132.18 ± 0.65**	262.31±8.23**
VI	Chloroform extract (400 mg/kg)	7.34±0.17** *	2.30± 0.26***	121.18± 0.64***	253.21±8.30***
XI	Ethanolic extract (200 mg/kg)	9.20± 0.14**	1.77 ± 0.25**	143.11 ± 0.59**	270.12±8.14**
XII	Ethanolic extract (400 mg/kg)	8.89± 0.14**	1.85 ± 0.30**	140.18 ± 0.27**	265.11±7.30**
XV	Glibenclamide (5 mg/kg)	6.17±0.43** *	3.79± 0.22***	89.62 ± 2.67***	233.76±9.34***

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated gro.

Non-insulin dependent diabetes mellitus (NIDDM) is a multifactorial disease, which is characterized by hyperglycemia and lipoprotein abnormalities. These traits are hypothesized to be responsible for damage to cell membranes through non-enzymatic glycosylation of proteins, auto-oxidation of glucose or increase metabolism of glucose by the sorbitol–polyol pathway. Cell damages will in turn, result in elevated production of reactive oxygen species or ROS. High levels of ROS have been found to play a role in the pathogenesis of NIDDM.[9]

Prolonged exposure to free radicals is a pivotal cause of tissue stress and injury. The free radical couses permanent damage to tissue structures results from an irreversible alteration in the molecular configuration of carbohydrates, lipids, proteins and even nucleic acid bases. In diabetes, the level of free radicals was reported to increase in alloxan and streptozocin treated rats an elevated level of free radicals was detected in several tissues including the kidneys.[10]

Conventionally, insulin dependent diabetes is treated with exogenous insulin and non insulin dependent diabetes with synthetic oral hypoglycaemic agents like sulphonylureas and biguanides. However, hormone fails as a curative agent for complications of diabetes and the major drawbacks of insulin therapy are the side effects like insulin allergy, lipodystrophy and lipoatropy, insulin antibodies, altered metabolic control, autoimmunity and other late complications like morphological changes in kidneys and severe vascular complications. Similarly, oral hypoglycaemic drugs have many side effects such as nausea, vomiting, cholestatic jaundice, aplastic and haemolytic anemia, generalised hypersensitivity reactions, dermatological reaction etc.

Traditionally, there are various herbs are being used for the treatment of diabetes mellitus, from which merely some have been evaluated as per the modern system of medicine. From these plants only plant extracts have been prepared and evaluated for its Antihyperglycaemic activity. Most of the reported plants seem to act directly on pancreas and stimulate insulin release in the blood. Some will favorably alter the activities of regulatory enzymes of glycolysis, gluconeogenesis and other pathways by acting directly on tissues like liver, muscle and fat (extra-pancreatic effect). Chemical constituents of these plants are known to possess wide range of medicinal properties.

The research was envisaged for antidiabetic activity of different extracts procured by successive extraction methods and to find out or isolate the most possible active compounds from the active extracts showing the best activity. The antidiabetic activity of all extracts has been evaluated by STZ induced diabetes. The isolated compounds have been evaluated by In- vitro and In-vivo models.

Total ash value asses the total amount of material remained after detonation and the amount of heavy metals and inorganic compounds present in the powder sample. The total ash content was 5 times greater than acid insoluble ash.

The water and volatile content of a crude drug were determined by test for loss on drying. High water content will deprecate phytochemical constituents followed by hydrolysis and enhance growth of microorganisms. Hence there should be a set of confines for water content for a plant under research. In our investigation the percentage yield of loss on dying was found to be 6.98% (w/w). Extractive values are chiefly used for the determination of exhausted or adulterated drug. The alcohol soluble extractives values were found to be higher than water soluble extractive value. Alcohol being a moderately non polar solvent, able to extract polar and non polar components yields higher extractive value.

Literature review states that the presence of alkaloids flavonoids, glycosides, terpenes, srteroids, polysaccharides, phenols, coumarins and proteins in the plant extract contribute to pharmacological activities such as antidiabetic, hypoglycemic, antihyperlipidemic and antioxidant properties **(**Stahl, 1969**)**. Preliminary phytochemical evaluation report illustrates that petroleum ether extract of leaves of CT showed the existence of triterpenoids, steroids and fatty acids, chloroform extract showed presence of saponins, phytosterols, flavonoids, phenols, steroids, terpenoids ethanolic extract showed the presence of alkaloids, flavonoids and glycosides and aqueous extract showed the presence of carbohydrates, as phytoconstituents.

Hence, keeping all this in view, research work was focused on the above-mentioned constituents in both extracts, for the evaluation of hypoglycemic, antidiabetic and antioxidant potential. Toxicity study of a new compound must be done accurately for the selection of the dose, used for its pharmacological screening. This study is carried out on animals in the laboratory with a very sophisticated procedure.

In this study, all the extracts at the dose of 2000mg/kg indexed neither visible signs of toxicity nor mortality and observations did not point out any proofs of substance related toxicity. The no-observed- adverse-effect level (NOAEL) was noticed at the dose of 2000mg/kg. The toxicity studies was determined by OECD guidelines 423.Based on the LD50 value, $1/5^{th}$ and $1/10^{th}$ (200 & 400 mg/kg) of its value was chosen for pharmacological study.

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