International Journal of Medicine and Pharmaceutical Research

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RESEARCH ARTICLE

Anti-diabetic Activity of Methonolic Extract of *Eugenia jambolana* Seeds and *Cinnamomum zeylanicum* bark on Alloxan Induced Diabetic Rats

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ABSTRACT

Eugenia jambolana (EJ) and *Cinnamomumzeylanicum (CZ)* are used comprehensively in the indigenous system of medicine as anti-diabetic agents. The aim of the present work is to study the combined action of Methanolic extract of *Eugenia jambolana* and *Cinnamomumzeylanicum (EJ+CZ)*on blood glucose levels in alloxan induced diabetic albino rats. Rats were injected intraperitonially with alloxan monohydrate at a dose of 150mg/kg to elevate the blood glucose levels. Rats with blood glucose levels more than 200mg/dl were considered hyperglycemic and were selected for the study. Following the treatment with combination of*(EJ+CZ)*at various doses of100mg/kg, 200mg/kg, 400mg/kg to the animals, the blood samples were collected and blood glucose levels estimated. Both the plants in combination, succeeded in lowering blood glucose levels in rats, moreover 28 days treatment with combination of both herbs significantly reduced blood glucose level. It can be concluded that *Eugenia jambolana* and *Cinnamomumzeylanicum* combination can be used as natural blood glucose lowering agent.

Keywords: Eugenia jambolana, Cinnamomumzeylanicum, Alloxan, Diabetes Mellitus.

ARTICLE INFO

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PAPER-QRCODE

ARTICLE HISTORY: Received 11 Sept 2018, **Accepted** 28 October 2018, **Available Online**10 December 2018

Citation: *K. Jyothsna Jayaraju, et al.* Anti-diabetic Activity of Methonolic Extract of Eugenia jambolana Seeds and Cinnamomum zeylanicum bark on Alloxan Induced Diabetic Rats. *Int. J. Med. Pharm. Res., 2018, 6(6): 278-283.*

CONTENTS

1. Introduction

Diabetes mellitus is subsequent from the Greek word "Diabainein" which means to "flow through". According to the W.H.O definition Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic glycaemia with disturbances of carbohydrate, fat and protein metabolism consequential from defects in insulin secretion, insulin action or both[1]. The fundamental goal of all diabetes treatment and management is to sustain an adequate blood glucose concentration[2]. Diabetes mellitus may be categorized in to several types but the 2 major types

are type I and type II. On the origin of etiology, the term type I and type II were extensively used to describe insulin dependent diabetes mellitus and non-insulin dependent diabetes mellitus correspondingly other specific type of diabetes and gestational diabetes are given in the term juvenile on set of diabetes has sometimes been used for insulin dependent diabetes mellitus and maturity diabetes for non-insulin dependent diabetes[3].

Type I diabetes (formerly known as insulin dependent diabetes mellitus –IDDM- or juvenile –onset diabetes): in type 1 diabetes, there is insufficiency of insulin due to autoimmune destruction of B cells. Insulin treatment is desirable for such patients as they are prone to diabetic ketoacidosis. Type I diabetic patients are usually young (children or adolescents) and not obese when they first develop symptoms. Type II Diabetes (formerly known as non insulin dependent diabetes mellitus- NIDDM- or maturity-on set diabetes): Type 2 diabetes is related with insulin resistance and impaired insulin secretion, both of which are important in its pathogenesis. Such patients are often obese and the prevalence rises gradually with age as B-cells function declines.

Type I diabetes is a multifactorial autoimmune disease due to rigorous insulin insufficiency that is influenced by environmental and genetic factors. Type II diabetes is non immune disorder with unreliabledegrees of insulin resistance and impaired insulin secretion usually allied with obesity. Diabetes mellitus can cause long term and short term complications if untreated. Among those complications diabetic neuropathy are the major complications[4]. In the last few years there has been an exponential expansion in the field of herbal medicine and these drugs are in advance popularity both in developing and developed countries because of their natural origin.

For centuries plants have been used to treat human diseases. In Ayurveda various herbs are reported for treating and preventing diabetes. Herbal drugs have lesser or no side effects and are less exclusive as compared to synthetic drugs. Therefore discovery and segregation of antihyperglycemic compounds from the plants have become more important. According to the W.H.O, other than 150 plants are known to be used for the management of diabetes mellitus and the study of hypoglycemic plants is then optimistic[5]. Cinnamon is one of the traditional herbs used as a therapy for diabetes.

Cinnamon extract has amendable role in type II diabetic animal models. It was found to diminish blood glucose in a dose dependent manner as well as triglycerides, total cholestrol[6]. In India decoction of kernels of *Eugenia jambolana* is used as household therapy for diabetes. This also forms a major constituent of many herbal formulations for diabetes. Antihyperglycemic effect of aqueous and alcoholic extract as well as lyophilized powder shows diminution in blood glucose[7]. Traditionally in Indian medicinal system it is always found that composite extract is most effective than the separate one. The present work attempts to study the Antihyperglycemic potency of *Eugenia jambolana* seeds and *Cinnamomumzeylanicum* bark as a composite extract.

2. Materials and Methods

Drugs and chemicals:

Alloxan, Glibenclamide, Glucose, Methanol, All the chemicals used are of standard qualities.

Plant Material

Collection and authentication: The seeds of *Eugenia jambolana* and bark of *Cinnamomumzeylanicum* were collected and the plant was identified and authenticated.

Preparation of extract

***Eugenia jambolana*:** The air dried seeds were powdered and 50g powder was extracted using methanol in a soxhlet apparatus and was evaporated to dryness under pressure in rotary evaporator. The yield of methanol extract was 14.6 g %. The dry residue of the crude extract obtained was stored at 4^o c for further use[8].

***Cinnamomumzeylanicum*:** Shade dried bark 50 g was milled and extracted using methanol in soxhlet apparatus for 8hours. Then extract was evaporated to dryness and the final dry chocolate colour crude extract was stored in dark at -20^0 c until used for evapouration[9].

Phytochemical Screening

A preliminary phytochemical screening of methanolic extract of *Eugenia jambolana and Cinnamomumzeylanicum* was carried out according to standard methods.

Experimental Animals

Male Albino rats weighing about 150-200 gm obtained from Sanzyme private limited Hyderabad are used for study. The animals were housed in cages and fed with standard pellets and water *ad libitum* and the animals were exposed to alternate cycle of 12 hrs of darkness and light each. The animals were kept at $25\text{-}30^o$ c and 45-55% relative humidity. All animals were carefully monitored and all the experimental protocols were approved by institutional animal ethical committee. For experimental purpose animals were kept fasting overnight but had free access in water.

Oral Glucose Tolerence Test

The animals were overnight fasted and then orally administered with 2.0 g/kg glucose. Blood glucose levels were measured at 0, 30, 60 and 120 min after glucose load[10].

Experimental Induction of Diabetes

A single dose (150 mg/kg,i.p) of alloxan monohydrate dissolved in normal saline was used for induction of type II diabetes in rats after overnight fasting. After 1 hr of alloxan administration, the animals were fed standard pellets and water *ad libitum*. The animals were stabilized for 72 hrs and animals showing blood glucose level more than 200 mg/dl were selected for the study[11].

Experimental Design

All the experimental animals were divided in to six groups as normal group, diabetic group, standard group, Test 1, Test 2, and Test 3. All the drugs were administered orally and treatment continued for 28 days. The doses employed for all drugs were in therapeutic range to suit the experimental animal used. Group 2,3,4,5 and 6 were

induced diabetic using alloxan 150 mg/kg, before drug treatment.

Table no 1: the animal groups and treatment schedule

GROUP	TYPE	TREATMENT
Group-1	Normal group	Normal saline
Group-2	Diabetic group	Alloxan 150mg/kg +normal saline
Group-3	Standard group	Alloxan150mg/kg+5mg/kg of Glibenclamide
Group-4	Test-1 (low dose)	Alloxan150mg/kg +100mg/kg of methanolic composite extract of *EJ* and *CZ*
Group-5	Test-2 (medium dose)	Alloxan150mg/kg +200mg/kg of methanolic composite extract of *EJ* and *CZ*
Group-6	Test-3 (high dose)	Alloxan150mg/kg +400mg/kg of methanolic composite extract of *EJ* and *CZ*

Measurement of Body Weight & Blood Glucose Level: The body weight and blood glucose level were measured on weekly basis (i.e. 0, 7,14 and 28 days). Blood samples were obtained by tail vein puncture of both the normal and alloxan induced diabetic rats. Blood glucose level was measured by single touch Glucometer.

Statistical Analysis:

All results were expressed as mean ± S.E.M and statistically analyzed by a one-way analysis variance was performed using Graph pad Prism 5 statistical software. Tukey's test was used for group comparisons. The values were considered significantly different when the p-value was lower than 0.05.

3. Results and Discussion

Phytochemical Screening: Phytochemical screening of extracts of *Eugenia jambolana* showed presence of alkaloids, glycosides, triterpenoids, steroids, saponins, flavonoids, tannins except carbohydrates in the extracts of *Eugenia jambolana* seed and phytochemical screening of extract of *Cinnamomumzeylanicum* showed the presence of steroids, alkaloid, saponins, tannins and phenol.

Effect of composite methanolic extract of *EJ+CZ*on OGTT: The blood glucose levels in normal animals were increased to maximum at 30 min by the administration of glucose (2gm/kg) and there after showed gradual decrease in blood glucose levels in 60, 90, 120 mins. Glibenclamide (5mg/kg), *EJ+CZ* 100mg/kg , *EJ+CZ* 200mg/kg and *EJ+CZ* 400mg/kg treated animals showed significant ($p < 0.001$) decrease in glucose levels compared to normal

Results are expressed as Mean ± S.E.M, All groups are compared with control. n=6.The body weights of alloxan control animals were decreased compared to normal control animals on 0^{th}, 7^{th}, $14^{th,}$ 28^{th}days. Glibenclamide (5mg/kg), *EJ+CZ* 100mg/kg, *EJ+CZ* (200mg/kg) and *EJ+CZ* (400mg/kg) treated animals showed significant ($p < 0.001$) increase in body weights compared to alloxan control animals on 0^{th}, 7^{th}, $14^{th}28^{th}$ days.

Effect of composite methanolic extract of *EJ+CZ*on fasting blood glucose levels: The fasting blood glucose levels were significantly ($p< 0.001$) increased in alloxan control group on 7^{th}, 14^{th}, 28^{th}days compared to normal control animals. Glibenclamide (5mg/kg, po) and *EJ+CZ*) 100mg/kg, 200mg/kg, 400mg/kg, treated animals showed significant ($p< 0.001$) decrease in fasting blood glucose levels compared to alloxan control group on 28^{th} days.

Discussion

Diabetes is a metabolic disorder coupled with hyperglycemia and impaired metabolic process of carbohydrates, proteins and lipids due to paucity of insulin or its action or both. The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over-production of glucose excessive hepatic glycogenolysis and gluconeogenesis and/or decreased consumption of glucose by the tissues. In Indian system of medicine, plants have been a major source of drugs for the treatment of diabetes mellitus. World health organization has suggested that, assessment of potential of plants as effective therapeutic agents, especially in areas where there is lack of safer modern drugs[12]. In modern medicine (insulin, Sulphonylureas, Biguanides and thiazolidine diones), no acceptable effective therapy is till available to cure the diabetes mellitus. The management of diabetes mellitus has been cramped to use of oral hypoglycemic agents and insulin, earlier being reported to possess serious side effects. This leads to mounting demand for herbal products with antidiabetic factor with little side effect. The present study is undertaken to examine the action of methanolic extract of *Eugenia jambolana and Cinnamomumzeylanicum* in alloxan induced diabetic rats.

The preliminary phytochemical screening of *Eugenia jambolana*showed the presence of alkaloids, glycosides, Triterpinoids, steroids, saponins, flavonoids, tannins. Cinnamomumzeylanicum shows the presence of steroids, alkaloids, saponins, tannins and phenols. OGTT is a preliminary screening performed in normal animals before administration of alloxan whether plant extracts having antidiabetic activity or not. OGTT revealed that Glibenclamide (5 mg/kg, po) and methanolic extracts from the *Cinnamomumzeylanicum* and *Eugenia jambolana* in composite manner (100mg/kg, 200 mg/kg and 400 mg/kg, po) extract treated animals showed significant decrease in blood glucose levels in at 60 min, 90 min and 120 min in glucose loaded animals compared to normal animals. The lowering of glucose levels is may be due to the inhibition of intestinal absorption, or it may act by potentiating the secretion of insulin and by increase in the utilization of glucose levels in muscles. Alloxan is most well-known chemical compound used in diabetogenic research. Alloxan is a urea derivative which causes selective necrosis of the β-cells of pancreatic islets. It has been widely used to

persuade experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used. The chemical name of alloxan is 2,4,5,6 tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone, which is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution.

Alloxan-induced diabetes was characterized by severe loss in body weight [13]. The dwindle in body weight in diabetic control rats showed that the loss or degradation of proteins was because of diabetes. Proteins are known to contribute to body weight. Hakim *et al* have reported that decreased body weight in diabetic rats is due to dehydration and catabolism of fats and proteins[14]. Increased catabolic reactions leading to muscle wasting might also be the reason for the reduced weight gain by diabetic rats[15]. Alloxan control rats showed that decrease in body weights compared to normal control rats. Glibenclamide (5 mg/kg, po) and composite extract of *CZ+EJ* (100mg/kg, 200 mg/kg and 400 mg/kg, po) treated animals showed enhance in body weight compared to alloxan control group, which may be due to it's protective effect in muscle wasting i.e. reversal of gluconeogenesis and may be also decrease in protein degradation by stimulating insulin release. The hypoglycemic activity of composite extract of *CZ+EJ*was compared with glibenclamide, a standard hypoglycemic drug. Sulphonylureas such as *glibenclamide* have been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic b -cells principally by inhibiting ATP-sensitive K (KATP) channels in the plasma membrane. Further, it was accomplished that sulfonylureas have a direct effect on beta cell exocytosis and that consequence is mediated by a mechanism that does not involve direct activation of protein kinase C, which plays a major role in controlling the beta cell potential. The inhibition of ATP sensitive channels leads to membrane depolarization, activation of voltage gated Ca channels, increased Ca influx, a rise in cytosolic Ca and thereby insulin release.

The *CZ* bark contains dimeric, trimeric, and higher oligomericproanthocyandins with doubly linked bis-flavan-3-ol units in the molecule[16]. In addition, the extracts of *C. zeylanicum* have also been shown to improve insulin receptor function by activating insulin receptor kinase and inhibiting insulin receptor phosphatase, leading to amplified insulin sensitivity. The oral administration of *EJ* seed extract decreased the blood glucose level in diabetic rats. Administration of medicinal plant extract to gently diabetic rats resulted in activation of b-cells and granulation returns to normal giving insulinogenic effect[17]. In diabetic control rats showed considerable increase in fasting blood glucose levels compared to normal control, Glibenclamide (5 mg/kg, po) and composite extract of (*CZ+EJ*)(100mg/kg, 200 mg/kg and 400 mg/kg, po) treated animals showed significant decrease in blood glucose levels compared to diabetic control rats. Thus this study showed that administration of Methanolic extracts of *CZ+EJ* in composite manner exhibited better antidiabetogenic activity than when compared to the individual extract of the plants. It can be suggested that the active antihyperglycemic agents present in the composite extract helps in overcoming the diabetic complications by increasing the insulin secretion.

Table 1: Effect of *EJ+CZ* on Oral Glucose Tolerance Test (OGTT)

S.No	Groups	Blood glucose levels (in mg/dl)			
		0min	**30min**	**60 min**	**120 min**
1	Normal control	102.4±0.50	122.4±0.67	111.6±1.20	95.80±0.37
2	Glibenclamide (10mg/kg)	96.60±0.50	102.8±0.58	94±0.54	85±0.54
3	EJ+CZ (100mg/kg)	98.20±0.37	110.8±0.73	105.4±1.53	96.20±0.37
4	EJ+CZ (200mg/kg)	98±0.54	107.6±0.50	104±1.14	95±0.24
5	EJ+CZ (400mg/kg)	98.4±0.40	105.2±0.37	96±1.04	88.6±0.50

Values were expressed as mean ± S.E.M of 6 observations

Table 3: Effect of various concentrations of methanolic composite extract of *EJ+CZ* on body weight in alloxan induced diabetic rats.

Group	Treatment	Body weight (in gm)				
		0^{th} day	**7^{th} day**	**14^{th} day**	**21^{st} day**	**28^{th} day**
I	Normal	183.6±1030	185.4±0.8718	188.2±1.356	191.4±1.122	194.6±1.327
II	Diabetic	159.8±0.6633	153.8±0.5831	145.8±0.7348	134.4±1.077	122.6±1.077
III	Standard	172.4±0.6782	177.4±0.8124	182±0.7071	187.4±0.8124	190.8±0.5831
IV	Diabetic+ *(EJ +CZ)* 100 mg/kg	162.8±0.602	166.0±0.7071	171±0.7071	175.2±0.6633	180.4±0.9274
V	Diabetic + *(EJ +CZ)*	164.6±0.5099	169.6±0.6782	174.6±0.5099	179.6±0.6782	185.6±0.6782

	200 mg/kg					
VI	Diabetic + (EJ +CZ) 400 mg/kg	168.6±0.5099	172.2±1.114	179.6±0.6782	183.6±0.5099	187.8±0.8602

Table 4: Effect of various concentrations of methanolic composite extract of *EJ+CZ* on blood glucose levels in alloxan induced diabetic rats.

Group	Treatment	BLOOD GLUCOSE LEVELS				
		0^{th} day	**7^{th} day**	**14^{th} day**	**21^{st} day**	**28^{th} day**
I	Normal	93.8±1.393	95±1.225	95.80±0.8602	96.40±1.122	98.0±1.304
II	Diabetic	393.4±0.9274	406±1.077	418.4±1.166	434.4±1.208	443.2±2.354
III	Standard	331.6±2.379	271.4±1.030	214.2±1.908	152.4±1.288	108.2±0.6633
IV	Diabetic + (EJ +CZ) 100 mg/kg	374.0±1.414	334.4±1.208	272±2.408	209.6±1.965	158.6±1.965
V	Diabetic + (EJ +CZ) 200 mg/kg	362.2±1.158	317±0.7071	260±1.643	194.6±1.327	140.4±1.503
VI	Diabetic + (EJ +CZ) 400 mg/kg	349.0±2.828	294.4±1.166	240.8±2.177	167.6±1.030	119.4±0.7483

Results are expressed as Mean ± S.E.M; All groups are compared with control. n=6

4. Conclusion

In the present investigation it was observed that the composite extract of *CZ+EJ*has shown significant decrease in blood glucose levels in rats due to their phytochemical constituents like alkaloids, glycosides, Triterpenoids, steroids, saponins, flavonoids etc. Further studies are needed to elucidate the components which are responsible for anti diabetic activity.

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