

Antidiabetic Activity of *Erythrina indica*

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Abstract: The aim of this research is to study the antibiotic activity of the plant *Erythrina indica* bark that makes this plant one of the medicinal plants which widely used in traditional medicine where is used to reduce the effect and complication of diabetes mellitus in many countries in the world. This study consist of two main steps, the first step is to induce diabetes in laboratory animals and he second step is to study the effect of the plant *Erythrina indica* bark extract on the laboratory animals.

Key words: Alloxan, diabetes mellitus, blood glucose levels, beta-cells, antibiotic, *Erythrina indica*

INTRODUCTION

Diabetes mellitus is a chronic condition that affects between 1 and 5% in all over the world according to, the prevalence estimation (Meral *et al.*, 2004). The majority of the diabetics about 85 of the diabetics they might have retinopathy and between (25-50%) are suffering from kidney conditions and about (60-70%) of the diabetics are subjected to the nerve damage. Chronic hyperglycemia of the diabetes mellitus is always associated with the long-term damage, dysfunction and various potential medical complications. The patients with diabetes mellitus have more probability to be subjected to have a stroke (Gabir *et al.*, 2000). The applications of the chemical compound Allaxon to different kinds of animals produces, via. necrosis of the islets, shows that many features common to that found in human diabetic patients (Lukenes has been reported that uncontrolled type I diabetics are more subjected to microbial infections than the normal controls and the cause of this phenomena is not known yet (Larkin *et al.*, 1985). The main goal of our research is to determine the histopathological additions which associate with the alloxan-induced diabetes in rats.

The oxidative stress and the changes in the antioxidant capacity that appear in the clinical and also the experimental diabetes condition are related to be the etiology of chronic diabetic mellitus effects (Ravi *et al.*, 2004). The complications that associated with diabetes mellitus include retinopathy with the probability of losing vision, neuropathy with risk of causing foot ulcers, amputations and charcot Joints and autonomic neuropathy that lead to gastrointestinal genitourinary and cardiovascular symptoms and sexual disability. The patients who have diabetes mellitus are subjected to potential risk of atherosclerotic cardiovascular, peripheral

arterial and cerebrovascular condition. The hypertension and the complications of lipoprote in metabolism are always appeared in the patients with diabetes mellitus disorder. Comparing with the synthetic drugs, the natural drugs that derived from the plants are always considered to have less toxic activity and side effects. Pari *et al.* (2001). And also it is characterized by the disorder of carbohydrate metabolism, persistent hyperglycemia, glucosuria and polyuria (Watkins, 2003; Brownlee, 2001). In all over the world, there are about 194 million individuals their ages between (20-79) years old suffer from diabetes disorder, this is estimated in 2003 and this number will be increased up to 333 million individuals around the next 20 years. So, the diabetes mellitus disorder is one of the pathological disorders which are associated with oxidative stress (Baynes and Thorpe, 1999; Ebuehi *et al.*, 2003).

The using of medicinal plants as a natural source of drugs and treatment, started at the time of immemorial, so. This natural source has been used in virtually all ancient and modern cultures as a natural source of medicine. And it may be counted as about 80-85% of population in the communities of both modern and developing countries depend on the traditional medicine for their elementary health care requirements and it may estimate as the main part of the traditional therapy include the using of plant extracts, products or their active principles (Kumar *et al.*, 2011).

Plan of work: The selected plant *Erythrina indica* was collected and authenticated. The bark of *Erythrina indica* are cleaned and should be made free from the dust particles and then should be dried in the shade and then made powder coarsely. After that the powder should be extracted by using ethyl acetate and methanol as solvent (increasing order of polarity) by soxhlet apparatus. The

resultant extracts were taken for the study. Initially, they were subjected to the preliminary phytochemical screening to know the phytoconstituents present. And after that the *in-vitro* studies, i.e., anti-inflammatory and antioxidant studies were carried out to determine which extract among the ethyl acetate and methanolic has the ability to show promising activity to proceed further for *in-vivo* antidiabetic and anti-inflammatory activities screening.

MATERIALS AND METHODS

Chemicals: The analytical graded chemicals were used for all the experiments.

Preparing the *Erythrina indica* bark: Take about 500 g of the powder of the plant *Erythrina indica* bark and this sample should be extracted by the use of soxhlet apparatus system with methanol and ethyl acetate for about forty 8 h at temperature of about 30°C. The extracts should be more concentrated by using the reduced pressure via. Rotary vacuum flash evaporator for making constant volume.

The phytochemical screening: *Erythrina indica* is not merely useful for the chemical compounds like carbohydrates, proteins and lipids which are used as a food by the human and also has very important compounds such as glycosides, alkaloids, gums, tannins, saponins and other important chemical compounds which can be used for its physiological and therapeutic activity. The majority of the chemical compounds which related to the medical activity of the products are kind of secondary metabolites. The plant material should be subjected to the preliminary phytochemical screening to detect the different plant constituents.

Alkaloids detection tests

Dragendorff's test: Add 1 mL of Dragendorff's reagent to the *Erythrina indica* bark extract. The appearance of the orange red colored precipitate shows the presence of alkaloids.

Wagner's test: Add 1 mL of Wagner's solution should be added to *Erythrina indica* bark extract. The reddish brown colored precipitate shows the presence of alkaloids.

Mayer's test: Add 1 mL of Mayer's solution should be added to *Erythrina indica* bark extract. The appearance of dull white colored precipitate shows the presence of alkaloids.

Hager's test: Add 3 mL of Hager's solution should be added to *Erythrina indica* bark extract. The appearance of yellow colored precipitate related to the alkaloids formation.

Tests for the carbohydrates

Molish test: Add 1 mL of a-naphthol solution to the *Erythrina indica* bark extract and then add the conc. Sulphuric acid on the sides of the test tube, the purple or the red-violet color appears on the junction that present between the two liquids related to the presence of the carbohydrate compounds.

Fehling test: Add the same amounts of Fehling solution A and B to the *Erythrina indica* bark extract by heating gradually, the appearance of the brick red precipitate shows that carbohydrate compounds are present.

Benedict test: Add 8 drops of the *Erythrina indica* bark extract to 5 mL of benedict's reagent and then the mixture should be boiling gradually for about 2 min and then should be cooled. The appearance of red precipitate shows the presence of the carbohydrates.

Protein detecting tests

Biuret test: Add 1 mL of 40% sodium hydroxide with two drops of 1% copper sulphate solution to *Erythrina indica* bark extract. The dark violet color shows the presenting of the proteins.

Xanthoprotic test: Add 1 mL of conc. Nitric acid to the *Erythrina indica* bark extract. When a white color precipitate appears, it should be boiled and then cooled and after that add 20% of sodium hydroxide. The appearance of the orange color shows the presence of aromatic amino acids.

Test for detecting the lead acetate: Add 1 mL of lead acetate solution to the *Erythrina indica* bark extract. The white precipitate shows presence of the proteins.

Amino acid detecting tests

Ninhydrin test: Add 2 drops of recently prepared 0.2% ninhydrine solution to *Erythrina indica* bark extract and then heat the mixture. The appearance of the blue color refers to the presence of proteins, peptides or amino acids.

The steroid detecting test

Libbermann burchard test: Dissolve the *Erythrina indica* bark extract in 2 mL of chloroform in a test tube. And then add 10 drops of the acetic anhydride and then add 2 drops

of con. sulphuric acid. The mixture becomes red and then turns blue, then after that the bluish green color will appear which refers to the presence of the steroid compounds.

Salkowasli test: Dissolve *Erythrina indica* bark in the chloroform and add the same amount of sulphuric acid to the mixture. The bluish red to cherry red color would be remarkable in the chloroform layer while acid layer that mark green fluorescence shows the presence of the steroids.

Test for the detection of cardiac glycosides

Keller-killani test: Dissolve the *Erythrina indica* bark extract in acetic acid containing trace of ferric chloride and transferred to the surface of con. sulphuric acid. The red-brown color would appear in the junction and then gradually would be blue to show the presenting of the cardiac glycoside compounds.

Test for the detection of saponnins

The foam test: Dilute 1 mL of the *Erythrina indica* bark extract in distilled water up to 20 mL and then shake it by the graduated cylinder for about 15 min. The appearance of the foam (About 1 cm layer of foam) shows the presence of the saponnins.

Invitro antioxidant activity (Reducing antioxidant power)

Principle: Presence of the normal antioxidants in the plants is well known. Plant phenolics which mainly attribute to the antioxidant activity are known to act by multiple biologic effects including redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers which also possess metal chelating potential.

Experimental procedure

Preparation of 0.2 M phosphate buffer:

- About 27.218 g of potassium dihydrogen phosphate was dissolved in 1000 mL distilled water to get 0.2 m solution
- About 8 g of sodium hydroxide was dissolved in 1000 mL distilled water to get 0.2 m solution

To prepare 0.2 m phosphate buffer, mix 50 mL of 0.2 m potassium dihydrogen phosphate with 16.4 mL of 0.2 m sodium hydroxide and make up the final volume to 200 mL with distilled water.

The ability of reducing the antioxidant of the *Erythrina indica* bark extract is determined by using the method of Oyaizu. Several concentrations of the *Erythrina indica* bark extract like 10, 100, 200 and 500 ug/mL were prepared. About 1 mL of these solutions of different concentrations is mixed with about 2.5 mL of

0.2 m phosphate buffer P^H 6.6 and 2.5 mL of 1% potassium ferri-cyanide [$K_3Fe(CN)_6$]. And then the about 20 min and after that 2.5 mL of trichloroacetic acid (10%) should be added to the mixture. And the centrifugation is used for about for 10 min at 3000 rpm. After that, the layer on the top of the mixture was taken (about 2.5 mL) and diluted by mixing with 2.5 mL distilled water and then 0.5 mL 0.1% $FeCl_3$ solution was added to the mixture. The absorbance measurement should be taken at 700 nm with the blank and the use of the UV-Vis spectrophotometer (Elico-SL 196). Any increasing in absorbance measurement of the solution related to the increase in reducing power.

Evaluation of anti diabetic activity

Toxicity studies: *Erythrina indica* bark extracts was given every day (400 mg/kg body weights of rats) by oral feeding tubes for a period of 3 weeks. The *Erythrina indica* bark extracts were given at the doses of 400 mg/kg/day of the body weight. All the animals found to be safe at the dose of 3000 mg/kg (as per OECD Guidelines).

The designing of experiment: The lab animals (rats) should be put into different groups.

Group 1: Consisted of 4 rats that are fed just regular food and keep them normal to use this group of rats as a regular control.

Group 2: Consisted of 2 alloxan induced diabetic rats and served as diabetic control.

Group 3: Consisted of 4 alloxan induced diabetic and this group of rats should get specific orally treatment with the Methanolic Extract of *Erythrina Indica* (MEEI) bark at a dose of 400 mg/kg body weight and this treatment should be daily regular treatment for 21 days as one dose every day.

Collection and processing of blood for estimation of blood

sugar levels: After 21 days of treating the rats regularly with the Methanolic Extract of *Erythrina Indica* (MEEI) bark, the experiments should be finished and the results should be observed. Body weight should be taken in consideration and should be checked before and after the experiment. The estimation of the glucose level of the blood should be taken at 0 day, 17, 14 and 21st day of the experiment by using the glucometer with strip method and the blood samples were taken from tip of the tail.

RESULTS AND DISCUSSION

Reducing antioxidant power: All types of the extract (methanolic and ethyl acetate extracts) of the plant

Erythrina indica bark should be tested to estimate the antioxidant activity. And according to, the results in Table 1 methanolic extract of the *Erythrina indica* bark have significant antioxidant activity whereas the ethyl acetate extract of the *Erythrina indica* bark has moderate antioxidant activity (Fig. 1).

The levels of glucose in serum, of alloxan induced diabetic rats were significantly elevated as compared with control rats. Oral administration of *Erythrina indica*

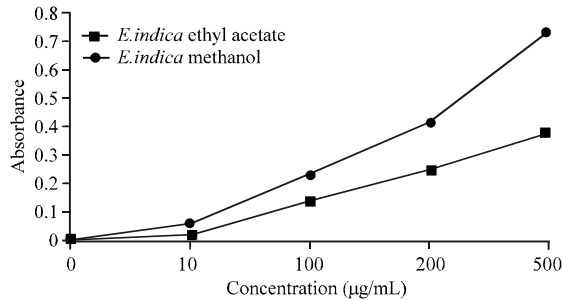


Fig. 1: Antioxidant activity of various extract concentrations of the *Erythrina indica* bark extract

(400 mg/kg of the body weight) to diabetic rats for 21 days resulted in obvious reducing in the serum glucose level as shown in Table 2-4.

Table 1: Preliminary phytochemical screening for various phytoconstituents

Test	Ethyl acetate extract	Methanolic extract
Carbohydrates (Benedict's test)	-	+
Proteins (Biuret test)	-	+
Amino acids (Ninhydrin test)	-	+
Alkaloids (Mayer's test)	+	+
Steroid (Salkowaski's test)	+	+
Phenolic compounds (CH ₃ COOPb)(FeCl ₃)	+	+
Tannins	+	+
Cardiac glycosides	+	+
Saponins	-	+

Table 2: Phytochemical screening reducing antioxidant power

Concentration	Absorbance	
	Ethyl acetate extract	Methanol extract
10	0.02	0.06
100	0.14	0.23
200	0.25	0.41
500	0.38	0.73

Table 3: Antidiabetic activity

1st dose/Body weights of sd rats (g)	Blood glucose levels of rats before induction of diabetes (mg/dL)	Concentration of alloxan given to induce given (120 mg/kg/g)	Body weights of sd rats after induction of diabetes (g) after 3 days	Blood glucose levels of rats after induction of diabetes after 3 days (mg/dL)
Normal/Control				
120	117	-	120	120
150	127	-	150	130
145	104	-	146	120
155	129	-	159	135
Alloxan injected				
160	143	19.2	180	191
150	108	18	155	170
Alloxan and plant extract treated				
175	114	21	170	167
170	128	20.04	160	216
150	126	18	160	130
200	95	24	170	High

Table 4: Moderate antioxidant activity

2nd dosage/body weights of sd rats after induction of diabetes (g)	Increasing concentration of alloxan given to induce diabetes (140 mg/kg)	Blood glucose levels of rats after induction of diabetes after 3 days (mg/dL/g)	Concentration of plant extract <i>Erythrina indica</i> bark given to rats (400 mg/kg/g) given every day	Blood glucose level after injection of plant extract to diabetic rats	Blood glucose level after injection of plant extract to diabetic rats
Normal/Control					
121	-	120	-	-	-
150	-	130	-	-	-
146	-	120	-	-	-
159	-	135	-	-	-
Alloxan injected					
180	25.2	400	-	-	-
155	21.2	high	Died due to high glucose level	Died due to high glucose level	Died due to high glucose level
Alloxan and plant extract treated					
170	23.8	437	68 mg (0.068 g)		
160	22.4	270	68 mg (0.068 g)		
160	22.4	576	Died due to high glucose level		
170	23.8	246	68 mg (0.068 g)		

The study found that the obvious reduction of the glucose level in *Erythrina indica* bark extract treated

diabetic lab animals (Sprague Dawley rats). The chemical compound alloxan creates a damage in the pancreatic a-cells and it also lead to a kidney damage that is kind of reversible damage but the streptozotocin selectively destroys the pancreatic insulin secreting a-cells (Gilman *et al.*, 1990). These changes in liver histology and kidney that caused by the alloxan activity are similar to the earlier observations (Shanmugasundaram *et al.*, 1983). Histologically, concerning to the liver section of alloxan induced diabetic rats there are obvious structural changes in the liver this is due to the lack of insulin. The significant alteration of this part was periportal fatty infiltration, necrosis of hepatocytes. This kind of damage is partially reversed by *Erythrina indica* (bark extract) treatment and is similar to that observed by *Gymnema sylvestre* therapy in alloxan diabetic rabbits by Shanmugasundaram *et al.* (1983). The present study was aimed to histopathological investigation *Erythrina indica* (bark extract) treated for the anti-diabetic properties in alloxan induced diabetes of S.D rats. Alloxan causes a massive reduction in insulin release by the destruction of the b-cells of the islets of langerhans, inducing hyperglycaemia (Fischer, 1985). The experimental diabetic animals (which induced by alloxan) exhibited an obvious elevation in BGL (Blood Glucose Level) after 7 days with the characteristic features of diabetes mellitus. The traditional plant medicines are used through all over the world for a range of diabetic presentations the study of such medicines might provide a natural key to disclose a diabetologist's pharmacy and using of medicinal plants as a natural source of drugs and treatment for the future. So, by working on this way, we are making a trial for the first time to study the effect of *E.indica* in normoglycaemic and hyperglycaemic rats. Both the extracts of *E.indica* were not able to decrease the BGL in the alloxan-induced diabetic lab animals (Sprague Dawley rats) on single dose administration which infers that the extracts are ineffective. The significant activity was attained on repeated administration of the methanolic extract from day 7 by controlling the elevated BGL compared with other group.

CONCLUSION

This study aims to show the evaluation of the significant effect of alloxan to create diabetes mellitus increase the blood glucose levels as well as it cause to damage liver, kidney and pancreas. Also to evaluate *Erythrina indica* (bark extract) (50% ethanolic extract) on causing the liver, kidney and pancreatic tissue damage or complications in alloxan-induced diabetic lab animals. The results of this study shows that the *Erythrina indica*

(bark extract) was actively leading to the improvement of liver, kidney and pancreas function and also reducing the lesions that associated with diabetic state in alloxan-diabetic rats. Beside the role of oral *Erythrina indica* (bark extract) at the dose 400 mg/kg body weight was more active.

SUGGESTIONS

From the present study it shows that these extracts can improve the glucose tolerance, suggesting that these extracts may show insulin mimetic activity or improved glucose utilization mechanism.

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