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Research Article

EVALUATION OF *IN VIVO* ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF *ARTOCARPUS HIRSUTUS* SEEDS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The objective of the study was to evaluate the antihyperglycemic activity and *in vivo* antioxidant effect of *Artocarpus hirsutus* seeds in both normal and diabetic rats.

Methods: Male Wistar rats weighing about 180-250 g were divided into six groups, of six rats each. Diabetes was induced by giving streptozotocin (30-50 mg/kg) intraperitoneally. Rats, which showed blood glucose levels ≥250 mg/dl, were selected for the study. Metformin (50 mg/kg) was used as a standard oral hypoglycemic agent. Oral glucose tolerance test was performed in all groups of rats. In the estimation of *in vivo* antioxidant activity, the levels of liver enzymes superoxide dismutase (SOD), lipid peroxidation, and CAT (catalase) were measured using standard methods.

Results: The ethyl acetate seed extract of *A. hirsutus* at different doses was selected and administered orally. The blood glucose levels were estimated by the glucose oxidase method, and insulin levels were measured by chemiluminescence assay method. Antihyperglycemic activity of the test drug in diabetic rats showed a significant reduction in blood glucose levels ($p<0.001$) at 2, 4, 6, and 8 hrs, respectively, as compared to diabetic groups. The antioxidant enzymes SOD and CAT levels were significantly raised, whereas malondialdehyde-thiobarbituric acid residue substances levels have decreased ($p<0.001$).

Conclusion: The results suggested that *A. hirsutus* seed extract showed a potential antidiabetic activity and antioxidant effect justifying the use of the drug for the treatment of diabetes mellitus and its associated oxidative damage.

Keywords: Antidiabetic, Antioxidant, *Artocarpus hirsutus*, Streptozotocin.

INTRODUCTION

Diabetes mellitus is recognized as one of the serious global health problems [1]. It is a group of metabolic disorders manifested by the presence of higher concentrations of glucose in the blood because of the improper production of insulin from the pancreas or inactivity of cells to the insulin [2]. It is also characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in enzymes and high oxidative stress, which induced damage to the pancreatic β islets [3]. There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by the generation of free radicals [4]. In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defenses [5]. Hence, compounds with antioxidative properties would be useful antidiabetic agents [4]. The treatment of diabetes mellitus in clinical practice has been confined to the use of oral hypoglycemic agents and insulin; the former being reported to be endowed with characteristic profiles of serious side effects. This leads to increasing demand for herbal products with antidiabetic factor with little side effects [6]. Drug treatment compliance is thus less than optimal and diabetes complications, therefore, also more prevalent. Thus, it is necessary that we look for a new and more efficacious drug in the herbal world. The present investigation is aimed to develop culturally adapted complementary and alternative therapies in traditional medicine to treat several symptoms of diabetes. The available literature shows that there are more than 400 plant species showing hypoglycemic activity [7].

The plants that show significant pharmacological activity and low toxicity need extensive screening. *Artocarpus hirsutus,* which belongs to the family Moraceae, is one of the ancient plants in the world. It is found in Western Ghats of India and Malabar Coast, mountain belts of Karnataka, Kerala, and Tamil Nadu [8]. It is useful in the treatment of anticancer, asthma, antibacterial and in the treatment of various skin diseases [9]. The chemical constituents include various flavonoids, phenolic compounds, and fatty acids [10].

The present study was undertaken by preparing the ethyl acetate extract of *A. hirsutus seeds* (EAEAH), and the resultant extract was evaluated for antidiabetic and antioxidant activity.

METHODS

Collection of plant material

A. hirsutus seeds (5 kg) were collected from Tirumala hills, Tirupathi, Andhra Pradesh, during the month of December 2015 to January 2016 and authenticated by Dr. Madhavasetty, Botony Department, S.V. University, Tirupathi.

Preparation of extract

The seeds obtained were powdered in the electric grinder, placed in closed vessels. To the powder, required amount of ethyl acetate is added and subjected to solvent extraction method.

Animals

Animal protocol was approved by institutional animal ethical committee (IAEC) of the CPCSEA (Committee for the purpose of control and supervision of experiments on animals) through its reference no. IAEC/SVCP/2011/007 dated 26/7/11. Male Wistar rats, weighing (180-250 g), were obtained from the National Institution of Nutrition, Hyderabad. The animals were housed with free access to food and water for at least 1 week in an air-conditioned room (25°C) under 12 hrs light-

dark cycle before the experiment. They were fed with standard diet (Hindustan Lever Pvt. Ltd.) and water *ad libitum.*

Acute toxicity studies

Acute toxicity testing is performed for the EAEAH following the OECD (Organization of Economic Cooperation and Development) guidelines - 420, different doses of extracts starting from 50, 100, 200, 500, and 1000, increasing up to 2000 mg/kg body weight was administered, and signs and symptoms of toxicity were observed for the next 48 hrs. No toxicity or death was observed in the experimental rats [11,12].

Antidiabetic activity

Induction of experimental diabetes

Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) solution (Sisco Research Laboratories Pvt. Ltd., Mumbai-93, India. Batch No.: T-835796) at the dose of 30 mg/kg in cold citrate buffer 0.1 M, pH 4.5 to overnight fasted rats. Control rats received only buffer solution. Diabetes was identified by polydipsia, polyuria, and by measuring non-fasting blood glucose levels 48 hrs after injection of STZ. Animals with blood glucose levels ≤ 250 mg/dl were not considered for the study [13].

Experimental groups

The animals were divided into seven groups of 6 animals each:
Group I: Normal untreated rats (control)
Group II: Diabetic control
Group III: Diabetic rats treated with metformin (50 mg/kg, p.o)
Group IV: Normal rats administered with (EAEAH) (100 mg/kg, p.o)
Group V: Normal rats administered with EAEAH (200 mg/kg, p.o)
Group VI: Diabetic rats administered with (EAEAH) (100 mg/kg, p.o)
Group VII: Diabetic rats administered with (EAEAH) (200 mg/kg, p.o).

Animals of Group I are administered with 0.9% saline and served as control. Group II were diabetic control rats; Group III received standard oral hypoglycemic drug metformin at the dose of 50 mg/kg. The drug was dissolved in sterile water and administered orally. Whereas Groups IV and V were given with ethyl acetate extract of seeds at doses 100 mg/kg and 200 mg/kg body weight. Diabetes-induced Groups VI and VII were given with ethyl acetate extracts of seeds at doses 100 and 200 mg/kg orally. The rats were treated about 10 days and on 11th day blood was withdrawn from orbital sinus puncture. Blood glucose levels were estimated by glucose oxidase method [14]. The serum insulin levels were estimated by chemiluminescence assay on the 11th day of the treatment [15].

Oral glucose tolerance test (OGTT)

The OGTT was performed in overnight fasted animals. After overnight fasting, a 0 minute blood sample (0.2 ml) was taken from each rat in different groups, viz., normal, diabetic, control, diabetic+metformin, normal+EAEAH (100 mg/kg), normal+EAEAH (200 mg/kg), diabetic+EAEAH (100 mg/kg), and diabetic+EAEAH (200 mg/kg). Test drugs were administered orally in 0.25% carboxymethylcellulose, and standard drug metformin was also administered orally to diabetic rats. Glucose solution (2 g/kg) was administered orally 30 minutes after the administration of the extracts. Blood samples were taken at 0, 30, 60, 90, and 120 minutes after glucose administration. OGTT is performed for all the groups. All the blood samples were collected with potassium and sodium fluoride solution for the estimation of blood glucose [16].

Antioxidant activity (Biochemical assays)

The liver was quickly removed, weighed, and homogenized in phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged at 1000 rpm, 15 minutes to remove debris. The supernatant was assayed the biochemical parameters such as malondialdehyde lipid peroxidation (LOP) [17], superoxide dismutase (SOD) [18], and catalase (CAT) [19].

RESULTS AND DISCUSSIONS

Plants have been used for several years as a source of traditional medicine to treat various diseases and disorders [20]. The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world. There is an increased demand for using natural products with antidiabetic activity, on the account of side effects associated with the use of insulin and oral hypoglycemic agents [21]. Phytochemical screening is an essential and very important part of medicinal plants research [22]. After the completion of the extraction process, the obtained ethyl acetate extract was identified for the presence of phytochemicals. Phytochemical screening revealed the presence of phenols, tannins, flavonoids, stilbenoids, benzofurans, and lectin called as jacalin.

The results obtained were evaluated. Diabetes was induced using streptozotocin at a dose of 30 mg/kg. The blood glucose levels were ≥ 250 mg/dl indicated the induction of diabetes. OGTT was performed in all rats from Groups I to VII (n=6), and the results are evaluated and shown in Table 1. The ethyl acetate extract-treated groups of diabetic rats showed a significant reduction ($p<0.0001$) in blood glucose levels at 0, 30, 60, 90, and 120 minutes, respectively.

Several animal experiment models have been in use to evaluate hypoglycemic activity, such as alloxan monohydrate and streptozotocin, Streptozotocin is a nitrosourea compound produced by streptomyces achromogenes, which specially induces DNA strand breakage in beta cells causing diabetes mellitus. There is no incidence of spontaneous reversion with STZ, and it is also observed that more than 90% of rats becoming diabetic [23]. Therefore, the STZ-induced diabetic model has been widely employed to induce diabetes in experimental animals. When there was a comparison of diabetic rats with the normal rats, there was increase in blood glucose levels significantly which showed that STZ produced a diabetogenic response in Wistar rats.

STZ-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage. Diabetes and animal experimental models exhibit oxidative stress due to persistent and chronic hyperglycemia which thereby depletes the activity of antioxidant defense system, and thus promotes denovo generation of free radicals.

Natural antioxidants present in the plants scavenge harmful free radicals from the body [24].

Currently available synthetic antioxidants such as BHT, butylated hydroxyl anisole, and tertiary butylated anisole and tertiary butylated hydroquinones are susceptible to cause negative health effects. Hence, strong restrictions have been placed, and there is trend to substitute them with naturally occurring antioxidants [25]. Several studies revealed that phenols mainly a type of flavonoids, from medicinal plants are safe and bioactive and possess antioxidant properties [26].

A continuous treatment was given to the specified groups for 10 days, and the blood glucose concentrations were observed on the 11th day. The antihyperglycemic activity of EAEAH seeds was evaluated. Extract-treated diabetic rats were compared to control diabetic rats and was found that there was a significant reduction in the blood glucose levels ($p <0.001$) at 2, 4, 6, and 8 hrs. When the same test drug-treated diabetic group was compared to standard drug-treated group, there was a significant decrease ($p<0.001$) in diabetic standard drug-treated groups at 1, 2, 4, 6, and 8 hrs, but the values of blood glucose levels were significantly less ($p <0.0001$) in metformin-treated diabetic rats, the results are shown in Table 2. Serum insulin levels were also evaluated in normal and drug-treated diabetic rats. The extract-treated diabetic rats, i.e., Group VII exhibited in significant raise in the serum insulin levels ($p<0.001$) when compared with diabetic control. The results are shown in Table 3. The EAEAH showed a significant decrease in the blood glucose levels as well as a raise in the insulin levels in the diabetic-

Table 1: Effect of *Artocarpus hirsutus* seed extract on OGTT in normal and diabetic rats

Treatment and dose (mg/kg)	Blood glucose levels (minutes)				
	0	30	60	90	120
Normal (control)	81.00±0.763	102.6±2.036	96.51±1.46	91.6±1.31	80.71±1.596
Diabetic (control)	296.83±44.43***	273.40±2.98***	296.7±3.35***	294.35±2.04***	270.08±1.54***
Diabetic+metformin	283.91±2.36NS	262.63±2.30*	245.34±3.23***	171.76±2.53***	129.41±2.72***
N+EAEAH (100 mg)	79.80±1.30	82.94±1.51	83.55±1.45*	83.77±1.20	81.60±0.77
N+EAEAH (200 mg)	89.73±1.77	80.13±1.05	82.28±1.39	84.34±1.86	80.15±1.35
D+EAEAH (100 mg)	298.24±3.34NS	276±1.91**	255.74±3.10***	178.60±1.83***	154±1.89***
D+EAEAH (200 mg)	287.01±3.22NS	252.31±2.62***	206±0.96***	188.84±2.57***	155.02±2.22***

Values are referred as mean±SEM. ***p<0.001: Extremely significant, **p<0.001: Highly significant, *p<0.01: Significant. EAEAH: Ethyl acetate seed extract of *Artocarpus hirsutus,* SEM: Standard error of mean

Table 2: Effect of *Artocarpus hirsutus* seed extract (EAEAH) on blood glucose levels in normal and diabetic rats

Treatment	0 hr	1 hr	2 hrs	4 hrs	6 hrs	8 hrs
Normal	93.63±3.26	85.48±3.19	93.4±1.51	84.90±1.18	85.86±0.66	82.75±1.01
Diabetic control	278.8±4.85**	277.41±2.93**	288.73±2.23**	287.02±1.92**	279.08±1.81**	270.4±1.16**
D+metformin	282.5±5.49	263.73±2.08	147.76±2.14**	136.33±1.83**	136.83±1.04**	127.66±0.84**
N+EAEAH (100 mg)	87.5±3.095	92.167±3.13	94.00±2.75	102.0±4.35	104±3.36	112±2.89
N+EAEAH (200 mg)	91±2.22	93.67±4.36	94.17±3.51	98.33±4.60	100±3.99	104±4.01
D+EAEAH (100 mg)	293±7.44	283±4.053**	269±4.02**	274.9±2.54**	270±1.36**	257.8±2.15**
D+EAEAH (200 mg)	299.51±8.40**	206.03±3.69**	174.41±5.96**	161.16±4.82**	152±0.95**	147.83±0.90**

Values are referred as mean±SEM. **p<0.001: Highly significant, EAEAH: Ethyl acetate seed extract of *Artocarpus hirsutus,* SEM: Standard error of mean

Table 3: Effect of *Artocarpus hirsutus* seed extract (EAEAH) on serum insulin levels in normal and diabetic rats

Treatment groups	0 hr	1 hr	2 hrs	4 hrs	6 hrs	8 hrs
Normal	13.6±0.32	13.7±0.313	13.52±0.255	13.8±0.37	13.6±0.19	13.7±0.17
Diabetic control	3.08±0.17	3.05±0.21	3.05±0.17**	3.10±0.27**	3.9±0.19**	3.23±0.21**
D+metformin	11.92±0.59	12.11±0.56**	12.23±0.20**	12.83±0.55**	12.15±0.56**	12.7±0.51**
N+EAEAH (100 mg)	12.89±0.31	13.08±0.33	14.06±0.24	13.34±0.26	12.78±0.19	13.54±0.18
N+EAEAH (200 mg)	14.36±0.43	13.08±0.38	13.56±0.36	13.29±0.31	12.97±0.24	13.52±0.17
D+EAEAH (100 mg)	3.90±0.38	3.65±0.26	3.62±0.26	3.16±0.32	3.15±0.19	3.80±0.25
D+EAEAH (200 mg)	4.64±0.51**	6.97±0.53**	7.56±0.02**	9.23±0.24**	10.93±0.52**	11.1±0.431**

Values are referred as mean±SEM. **p<0.001: Highly significant. EAEAH: Ethyl acetate seed extract of *Artocarpus hirsutus*, SEM: Standard error of mean

treated rats, suggesting that *A. hirsutus* seed extract possesses glucose lowering property, and also it increases peripheral utilization of glucose in the body which proves it to be a potential antihyperglycemic drug.

The *in vivo* antioxidant enzymes were measured in the liver. It was found that the extract-treated group showed significant raise in the CAT and SOD levels (p<0.001) and decreased in malondialdehyde. The results are shown in Table 4.

Oxidative stress in diabetes may partially be reduced by antioxidants and as seen antioxidants have been prescribed to reduce the long-term complications seen in diabetes. Although there are several drugs designed and targeted for the disease and disease complication, many possess side/toxic effects such as hepatotoxicity or cardiac failure. Although there are drugs which were reported to control blood glucose levels of diabetic subjects or animal models, there failed to control complications of diabetes. The present study has indicated the fact that the plant *A. hirsutus* antidiabetic and antioxidant constituents. Production of a safe antidiabetic drug is very much possible from the seed.

Table 4: Effect of *Artocarpus hirsutus* seed extract (EAEAH) on antioxidant enzymes levels in normal and diabetic rats

Treatment groups	LPO	SOD	Catalase
Normal	15.1±1.44	31.34±1.40	20.4±1.79
Diabetic	47.6±1.52**	19.00±0.670**	4.3±1.28**
D+metfotmin	17.8±1.56	48.11±0.653	11.93±0.865
N+EAEAH (100 mg)	14.78±0.56	32.00±1.46	21.5±1.80
N+EAEAH (200 mg)	15.9±1.32	30.08±1.39	19.61±1.52
D+EAEAH (100 mg)	3.11±2.24*	21.95±0.350*	7.01±0.48*
D+EAEAH (200 mg)	24.1±1.09**	34.2±0.925**	7.86±0.399**

Values are referred as mean±SEM. **p<0.001: Extremely significant, *p<0.01: Significant. LPO: Lipid peroxidation, SOD: Superoxide dismutase, EAEAH: Ethyl acetate seed extract of *Artocarpus hirsutus*, SEM: Standard error of mean, CAT: Catalase

CONCLUSION

Therapeutic potential of medicinal plants is mainly due to their phenolic compounds present in fruit, vegetables, nuts, seeds, stems, and flowers. Hence, medicinal plants are used by humans since the beginning of human life on earth. Our study has shown that different doses of *A. hirsutus* exhibited a significant antihyperglycemic activity in diabetic animals. As well as it has strong *in vivo* antioxidant potential. Thus, consumption of *A. hirsutus* could be helpful in improvement of the hyperglycemic condition and prevent diabetic complications. More detailed studies such as antihyperlipidemia and *in-vitro* antioxidant activity of the same are being carried out and may be required to understand the clear and detailed mechanism of action responsible for the antidiabetic activity.

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