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ANTIDIABETIC POTENTIAL OF ECLIPTA ANGUSTATA LEAVES IN RATS

Lucky Ali[1], Shreya Singh[2] and M. K. Gupta[3]

[1,2,3]School of Pharmacy, Career Point University, Kota-325003, Rajasthan, India

Abstract

Plants have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive. In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. Only preliminary studies have been reported for the antidiabetic activity of the leaves of *Eclipta angustata*. Therefore, an attempt has been made to evaluate the antidiabetic potential of the ethanolic extract of *Eclipta angustata* leaves in streptozotocin induced diabetic models. It was found that ethanol extract of the leaves of *Eclipta angustata* was active in STZ models in Sprague-Dawley(SD) rats.

Introduction

Plants have been integral to human life since the beginning of time, providing numerous essential products. They are the foundation of all life on Earth and a critical resource for human well-being. Every food we consume comes directly or indirectly from plants, with approximately 7,000 different plant species having been used as food throughout history. Diabetes is a condition characterized by abnormal metabolism of carbohydrates, proteins, and lipids, resulting in high blood sugar levels due to low insulin levels or insulin resistance. It is one of the top five causes of death worldwide, affecting about 150 million people today—a number nearly five times higher than a decade ago—and this figure may double by 2030. India has the highest number of diabetics in any country. The incidence of diabetes is expected to rise rapidly due to increasing obesity and lower activity levels (Sarwar N, et al., 2010).

In many Asian countries, including India, traditional medicines, especially those derived from plants, are commonly used to help manage diabetes. India has a rich tradition of using various potent herbs and herbal components for diabetes treatment (Papadakis MA 2002). Medicinal plants are valuable due to their diverse metabolites, including aliphatic and aromatic compounds, and functional groups that can alter metabolic pathways. These herbal medicines are often prescribed because they are effective, have fewer side effects, and are relatively low cost. Therefore, investigation on such agents from traditional medicinal plants has become more important. ***Eclipta angustata,*** a traditional herb from the Asteraceae family, is well-known for its numerous biological benefits and has been used extensively in traditional medicine. This plant is commonly found in regions such as India, China, Taiwan, and Japan (Bunn et al 1978). While it is considered a weed in some areas, it is also used as a natural hair dye and as a nutritious vegetable (Skov J et al 2020).

Eclipta angustata is a creeping plant that can grow up to 3 meters in height with long stems. It has white, solitary flowers that are 6 to 8 mm wide and lance-shaped, sessile leaves. The roots are cylindrical and grey, while the seeds are black, rounded, and have a wrinkled surface. Key distinguishing features include its sessile leaves, subovate achenes, and noticeable tubercles.

The primary aim of this study is to demonstrate the hypoglycemic activity of *Eclipta angustata* leaf extract. The study seeks to evaluate the efficacy and potency of the leaf extract in lowering blood sugar levels. The leaves were tested for their antidiabetic effects in an in vivo study using STZ-induced diabetic rats, with metformin serving as the standard hypoglycemic drug for comparison.

Materials and methods

Collection of Medicinal Plant

The ***Eclipta angustata*** were purchased from the local herbal market and the authentification was done by the Institute.

Extraction:

The air-dried leaves (1.0 kg) were cut into small pieces and placed in a glass percolator with 5 liters of a 1:1 ethanol and distilled water mixture. The mixture was left to stand at room temperature for 24 hours. The combined percolate was then concentrated under vacuum using a rotary evaporator at 40°C, yielding 31 grams of extract. This extract was used for the antidiabetic screening activity.

Experimental Animals:

Experimental protocols were approved by the Institutional Ethical and Usage Committee, Adult Sprague Dawley male rats, weighing 145–225 g were used in this study. Animals were housed in raised bottom mesh cages to prevent coprophagy and were kept in environmentally controlled rooms with temperature regulated at 22 ± 2◦C, 12/12 h light and dark cycle rotation.

Treatment groups for diabetic model:

Rats were divided into three groups.

Group I (Control group): Control group of animals were treated with 1% CMC, for 21 d after induction of diabetes in rats.

Group II (Treatment groups): Rats were treated with herbal extract *Eclipta angustata*. Rats administered with STZ (45 mg/kg body weight) intraperitonally. Oral administration of *Eclipta angustata* leaf extract (200 mg/kg body weight) in STZ induced rats from Day 3 to Day 21.

Group III (Reference drug treated): Rats were treated with marketed at metformin (100mg/kg) after 72 hr of streptozotocin treatment.

Preparation of Streptozotocin induced antidiabetic rat model

Male Sprague Dauwley rats of average body weight 145 ± 225 g having blood glucose profiles between 70-90 mg/dl were selected and administered streptozotocin (45 mg/kg body weight) to make them diabetic. The rats were allowed for 5% glucose solution to overwhelmed the drug induced hypoglycemia. After 72 h of administration of STZ, via tail pinch blood samples were

collected and glucose levels were estimated by glucostrips (One Touch Glucometer, Life Scan, Europe). Animals having fasting blood glucose levels above 200 mg/dl were considered diabetic and subsequently used in the present study.

Statistical analysis:

Data were expressed as mean ± S.E.M. Analysis was performed using Prism version 5.0 software, employing one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

This paper explains evidence based-information regarding the antidiabetic activity of ethanolic extract of *Eclipta angustata* in STZ induced models.

Figure 1: Representative photographs of ***Eclipta angustata***

Evaluation of anti-diabetic effect of Eclipta angustata against streptozotocin induced diabetic rat model:

Extract of *Eclipta angustata* (EA) at graded doses (100, 200 and 400 mg/kg, p.o.) were used and 200 mg/kg showed maximum reduction of glucose level in comparison of others dose. Whereas the metformin (reference drug) reduced the upraised glucose level due to the streptozotocin at 45 mg/kg, i.p. From this observation 200mg/kg dose of EA was identified as the effective dose and selected for further studies.

Whereas **Table 1** depicts the treatment schedule and its effect on STZ induced diabetic rats. This study was divided into four group in first group control, it was treated with 1% CMC only and not treated with STZ so the glucose level was normal for the last 21 days. Whereas, glucose level was high in 2nd group STZ only for last 21st days. Furthermore, in EA treated group

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glucose level were not as much high as in STZ only group, it means the EA had potential to reduce the upraise level of glucose. In addition, the reference drug metformin significantly reduced the glucose level after treatment of STZ.

Treatment	Glucose mg/dl			
		Treatment start after 3rd day of STZ		
	24 hr	3 day	10 day	21 day
Control 1% CMC	90.3±08.32	92.7±6.02	89.00±06.23	95.41±09.76
STZ (45 MG/KG)	257.87±06.4	219±28.43	241.0±22.47	238.5±33.21
EA (200 mg/kg)	189.5±30.89*	101.8±66.48	97.50±21.35*	100.20±8.22*
Metformin (100 mg/kg)	88.7±19.28	96.1±37.11	78.30±13.20*	72.50±9.918*

Table 1: Effect of ***Eclipta angustata*** (EA) extract and reference drug **Metformin** (Met) on control group after induction of diabetes. glucose level was monitored after 24 hr, 3rd day, 10th day and 21st day of STZ treatment. (n= 6 in each group). *Statistically significant at P<0.05 and **P< 0.01, in comparison to control. n = 6 in each group.

The histological studies were carried out to prove the efficacy of EA leaves extract. **Figure 1** is the photographs of histopathology of liver cells. In image A it depicts a normal liver cell structure. Whereas Image B is STZ-induced diabetic rat and we observed sinusoidal dilation, congestion of portal vessel and hemorrhagic of the liver cells. Further in Image C we found resorted liver cells after the treatment. q

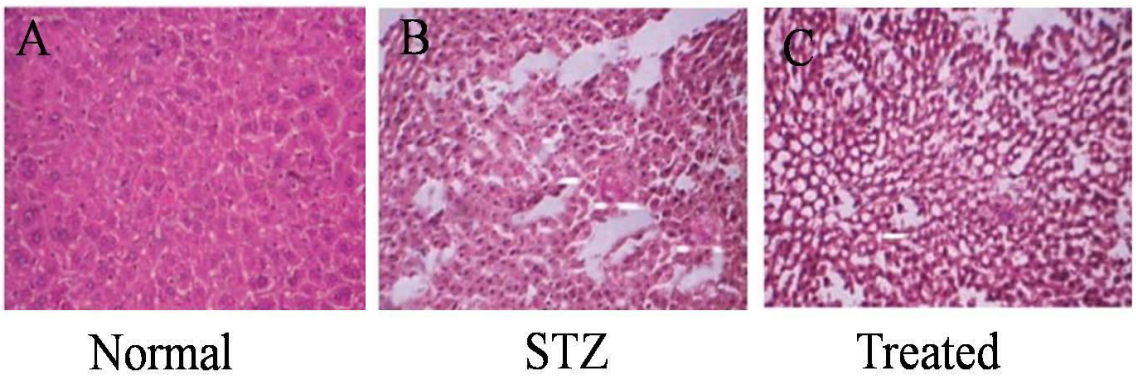

Figure 2: Histopathological photographs of rat Livers of streptozotocin induced diabetic models

Table 3 represents the effect of EA on the serum TG, Cholesterol, HDL and LDL levels. After the treatment of EA triglyceride level were reduced and also a sharp reduction in serum triglycerides concentration after the treatment of reference drug. Furthermore, EA also reduced the cholesterol and LDL but EA upregulate the level of HDL. This data exhibits the potent effect of EA on diabetes models.

Treatment	TG mg/dl	Cholesterol mg/dl	HDL mg/dl	LDL mg/dl
Control 1% CMC	398.5±66.48	278.6±33.34	58.00±08.48	75.00±13.76
EA (200 mg/kg)	314.5±30.89*	259.8±66.48	97.50±21.35*	58.20±8.22*
Metformin (100 mg/kg)	345.7±19.28	266.1±37.11	78.30±13.20*	72.50±9.918*
Fenofibrate (100 mg/kg)	261.2±22.01*	251.1±29.89	100.1±11.09*	52.50±11.84 **

Table 3: Effect of ***Eclipta angustata*** (EA), **Metformin** (Met) and **Fenofibrate** (Ffb) on glucose, Triglyceride (TG), High density lipid (HDL) and Low density lipid (LDL) contents in streptozotocin (STZ) induced diabetes model (n= 6 in each group).*Statistically significant at P<0.05 and **P< 0.01, in comparison to control. n = 6 in each group.

Anti-oxidant effect of Eclipta angustata:

The exquisite and consistent potency of compound EA in different experimental diabetic models prompted to choose it for further investigation. Antioxidants can reduce the oxidative stress and consequently, diminish the progress of stress related diseases (Mishra V et al., 2013). Thus, the antioxidant study of compound EA was carried to find out its effect on free radicals and reactive oxygen species. Results revealed that compound EA had the property to scavenge the free radicals and reactive oxygen species in a dose dependent manner in DPPH (1,1-diphenyl-2-picryl-hydrazyl) and Superoxide dismutase (SOD) assays as discussed below. The potent antioxidant activity exhibited by the EA has beneficial implications to play a role in the relief of the oxidative stress and long- term chronic complications.

In vitro anti-oxidant property of Eclipta angustata:

The EA exhibited strong antioxidant activity in the DPPH inhibition assay as evidenced by the low IC_{50} values 71.22 ±1.72 µg/ml (Table 4). Whereas, the positive control ascorbic acid showed 38.87±3.10 µg/mL in the DPPH inhibition assays.

Concentration (µg/ml)	1,1-diphenyl 1-2-picryl-hydrazyl (DPPH)
10	**10.73 ± 1.24**
20	**27.70 ± 3.59**
40	**35.91 ± 4.10**
60	**48.06 ± 8.16**
80	**53.87 ± 12.73**
100	**70.25 ± 10.08**
IC_{50} of EA	**71.22 ± 1.72**
Reference (Ascorbic acid)IC_{50}	**38.87 ± 3.10**

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Table 4: Values are expressed as percentage mean of 3 replicates

In vivo anti-oxidant property of Eclipta angustata:

Superoxide dismutase (SOD) was assayed according to Misra and Fridevich (1972) based on the inhibition of epinephrine auto-oxidation by the enzyme. In the control group the SOD activities were 0.079 ± 0.35(U/mg protein) after the treatment with ***Eclipta angustata*** it increases the SOD activity 0.312 ± 0.84 (U/mg protein) similar to reference antioxidant ascorbic acid treated group's SOD 0.346 ± 0.21 (P <0.01, when compared with control).

Discussion

Natural products brought substantial contributions to drug innovation by providing novel chemical structures and/or mechanisms of action (Cooke DW et al., 2008). In India; a large number of herbal extracts are used in folk medicine to treat various types of disorders. The present study has been conducted to evaluate the anti-diabetic activity of *Eclipta angustata* extract against STZ models of experimentally induced diabetes and to evaluate its mechanism of action involved in impediment of hyper/hypoglycemic development.

A preliminary study for the dose fixation of *Eclipta angustata crude* extract was conducted and 200mg/kg was found to be the optimum dose that can give the highest protection. The screening results were summarized in the table 1. Streptozotocin-induced diabetic murine models develop type 1 diabetes, due to the cytotoxic glucose analogue streptozotocin (STZ) that is toxic to pancreatic β-cells and causes insulin deficiency. STZ methylates DNA, causing DNA fragmentation and killing pancreatic β-cells. STZ related animal experiments appear to be a very good mimic of the human condition and have allowed studies into pathogenic mechanisms as well as useful therapeutic intervention (Lakshmi et al 2018)

. Thus, the significant protection imparted by EA in this model reflected the possibility of its involvement in the regulatory mechanism of diabetes. This interesting finding in STZ model intrigued to further explore its effects on other blood parameters of diabetic models in rats.

The effects of the EA on STZ induced and glucose level was examined and compared with the reference drug metformin (Table 3). Insulin and glucose plays a central role in diabetes. It is well known that reduction in insulin causes hyperglycemia. STZ has widely been used to induce diabetes in animals. It is a β-cell-specific toxin that induces irreversible damage to pancreatic islets through free radical generation and DNA damage. Besides, nitroso-containing STZ also releases nitric oxide that causes apoptosis (Keny 2014).

Antioxidants can reduce the oxidative stress and consequently ameliorate the progress of stress related diseases. An *in vitro* and *in vivo* antioxidant study of EA was carried out to find out its antioxidant properties. Results revealed that EA had property to scavenge the free radicals and reactive oxygen species in a dose dependent manner in DPPH and SOD assays. The antioxidant activity of the EA was suggested to play a role in the relief of long-term complications and the oxidative stress.

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Conclusion

This study provides evidence-based information on the antidiabetic activity of ethanol extract of *Eclipta angustata* (EA) in STZ-induced models. The results clearly indicate that the ethanol extract of EA has a beneficial effect. In conclusion, the leaves of EA inhibit the development of diabetes in rats by reducing glucose levels through the modulation of insulin hormone. Additionally, EA exhibits antioxidative properties by scavenging free radicals and reactive oxygen species. Due to its antidiabetic activity, EA may be a potent therapeutic agent for treating diabetes.

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