ANTI DIABETIC ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF ANDROGRAPHIS PANICULATA ON ALLOXAN INDUCE DIABETIC IN RAT

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ABSTRACT

Aims: The anti-diabetic effect of ethanolic leaves extract of Andrographis paniculata was inspected on Albino rats. Place and Duration of study was carried out between October 2020- January 2021 in the pharmacology Laboratory at Bharat Technology, Uluberia, Howrah, West Bengal, India. Methodology: Diabetic condition was introduced with an intraperitoneal injection of 120mg/kg BW alloxan monohydrate in the rats. After stable diabetic condition, the rats were administered with ethanolic extract of Andrographis paniculata for 10 consecutive days. Blood glucose profiles in both preprandial and postprandial were monitored at the day of 0, 3, 7and 10. In the study, the diabetes of the rat may decrease after administration of the herbal extract of A. paniculata. The extract may display significant hypoglycemic effects. In conclusion, the ethanolic extract of A. paniculata is potential to improve as an antidiabetic agent.

Keywords: Andrographis Paniculata Leaves, Andrographiloid, Diabetes, Alloxan Induce Diabetes.

I. INTRODUCTION

Diabetes is a disease in which the body's respond to the hormone insulin is impaired, causing abnormal metabolism of carbohydrates as a result of which glucose level elevated in the blood. Three types of diabetes are there:

1. Type 1- It is chronic medical condition where the pancreas produces small or no insulin
2. Type 2- It is chronic medical condition that the body becomes resistant to insulin
3. Gestational diabetes - This condition is due to insulin-blocking hormones produced during pregnancy. \[1\]

According to the IDF (International Diabetes Federation) report, India, china and United states have higher rates of most cases of Diabetes. Around 470 million adults (18-80 years) worldwide were living with Diabetes. The total number of diabetes patients is likely to be more than double in 2040. \[2\]

Alloxan which is an organic compound also known as 5, 5-dihydroxyl pyrimi-dine-2, 4, 6-trione, a urea derivative, cytotoxic and carcinogenic glucose analogue. The molecular formula of this compound is C\(_4\)H\(_{13}\)N\(_2\)O\(_4\) and a relative molecular mass of Alloxan is used to introduced diabetes in mice. \[3, 4\] Kalmegh (Andrographis paniculata) is commonly known as "king of bitter" and it is belonging in family Acanthaceae. It is used to treat a variety of infectious and chronic diseases as well as Diabetes. A. paniculata is one of the most commonly used medicinal plants and it is found in part of Asia and European countries. This local plant that are potentially developed as antidiabetic agents. \[5\] This study was aimed to explore antidiabetic effect of herbal extract of A. paniculata in alloxan-induced mice. Diabetic condition was introduced with an intraperitoneal injection of 120mg/kgBW alloxan monohydrate in the mice. After constant diabetic condition, the mice were administered with the extract for 10 consecutive days. Andrographis paniculata is known for its curative and preventive properties as whole plant is used such as stem, roots and leaves, which is the source of several diterpenoids of which andrographolide is important constituent which shows the Antidiabetic activity. Recent experiments have also shown that it's has some antibiotic and antityphoid activity. The aerial part and leaves of plant are used in treatment of diabetes, jaundice, liver disease, fever, diabetes, snake bite, dysentery, sore throat and chronic malaria. \[4\]

In India, the whole plant is used to acquire andrographolide, the most important pharmacologically active compound. Having such a broad geographical distribution and important medicinal value of this plant all over the country, its undiscriminating collection from wild sources without compensating any attention towards its
domestication and conservation in steady agriculture has caused a sharp drop in the availability of drug to the industries and acceleration in its prices. Therefore, the dense demand of phytochemicals especially diterpene lactone like andrographolide in India as well as international markets has encouraged Indian farmers to begin commercial cultivation of kalmegh, [5, 6]

II. MATERIALS AND METHOD

Plant material collection
The sample were collected from rural belt of Purba Medinipur, West Bengal, India in the month of September 2020. After collection of Plants, it is authentified by Pharmacognosist Dr. Sanjit Das, Asst. Professor, Department of Pharmacognosy, Bharat Technology, Banitabla, Uluberia, Howrah, West Bengal, India. The plant was separated from undesirable materials, cleaned, washed with distilled water and shade dried in room temperature and then powdered.

Preparation of extract
The shade dried leaves were powdered using a mechanical grinder and passed through 40 mesh sieves. Powder 300gm of powdered of leaves was successively extracted with 1.5 L of petroleum ether, chloroform and ethanol, in a soxhlet apparatus at 60–70°C each for 10–12 h consecutively. Solvents used were of analytical grade. Ethanol was removed from the extract by simple distillation and Evaporated to dryness and a semisolid mass was obtained. The semisolid mass was collected carefully sand packed in eppendorf, stored in refrigerator until use. Now the extracts were subjected to preclinical screening.

Experimental animal
Adult male and female, non-pregnant albino rats (average weighing 160 to 250 gm respectively) were used in this study. The animals were obtained from the department of pharmacology of Bharat Technology, Uluberia, Howrah, West Bengal, India. They were maintained under standard environmental condition (24 ± 2 °C, 60 to 70 % RH) in animal house approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) and fed with commercial pellet diet and water and libitum. They were taken out to the laboratory environment for at least two weeks before experiment. The Institution of animal ethics committee, Bharat Technology, Uluberia, Howrah, West Bengal, India approved the experiment protocol and following the guidelines and procedures of the" Principle of laboratory animal care" (National Institute of Health- NH publication number 85-23). [1, 7, 8]

Experimental Protocol
The animals will randomly be assigned into four groups of 6 in each group and received the treatments:

- Group I: Normal control rat will be treated with vehicle alone.
- Group II: Diabetic control rat will be treated with Alloxan. (120 mg/kg i.p)
- Group III: Diabetic rat will be treated with Glimipride. (5mg/kg i.o)
- Group IV: Diabetic rat will be treated with Andrographis paniculata extract. (400 mg/kg i.o)

All the 3 groups except normal control were injected with alloxan and were fasted for 18 hours. Then standard drug and A. paniculata extract were given orally to the rats group at the interval of 3, 7, 10 days. Check the blood sugar level of the rats at 2 days interval. [1]

Statistical analysis of data
The data obtained were examined by using the Graph pad Prism, version 9.1.1. All the values were presented in the table, they expressed as mean ± standard error mean (SEM) of six animals. The important difference between the mean diabetic index of treated group and that of the control group was tested with one-way analysis of variance (ANOVA) followed by Dunnett’s post-test and p values <0.05 deliberated significant. [8]

III. RESULTS

Phytochemical screening
Analysing of various chemical compounds within the extract, represents the preliminary phytochemical studies. Minor quantity of ethanolic extracts of Andrographis paniculata were subjected to preliminary quantitative phytochemical investigation for detection of phytochemicals like Tannins, Flavonoid, Alkaloid & Glycosides, Phenolic compounds & Tannins, Saponins, Steroid, Terpenoids, [8, 9],
Pharmacological study

A. Effect of administration of Alloxan (120mg/kg; i.p.) in rat.
The glucose level of Alloxan treated rat in ‘0’ days (before induced), ‘3’ days (after induced) ‘7’ days and ‘10’ days were found to 89± 2.83, 210± 6.37, 270± 3.40, 274± 1.78. Thus, Alloxan increases the level of glucose against vehicle treated (group I).

B. Effect of administration of Glimipride (5mg/kg orally) in Alloxan induced diabetic rat.
The glucose level of Glimipride treated rat in ‘0’ days (before induced), ‘3’ days (after induced), ‘7’ days and ‘10’ days are found to 92± 1.86, 190± 13.26, 154± 2.31, 98± 2.90. Thus, Glimipride significantly decrease the levels of glucose against Alloxan induced (Group II).

C. Effect of administration of ethanolic extract of leaves of Andrographis paniculata (400mg/kg orally) in Alloxan induced diabetic rat.
The glucose level of Andrographis paniculata treated rat in ‘0’ days (before induced), ‘3’ days (after induced), ‘7’ days and ‘10’ days were found 86± 2.13, 182± 3.86, 110± 2.49, 81± 2.26. Thus, Andrographis paniculata significantly decrease the level of glucose against Alloxan induced (Group II).

Table -1: Effect of ethanolic extract of the leaves of Andrographis paniculata on Alloxan induced diabetic rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>'0' days</th>
<th>'3' days</th>
<th>'7' days</th>
<th>'10' days</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>86±1.96</td>
<td>88± 2.01</td>
<td>90± 1.96</td>
<td>87 ± 1.47</td>
</tr>
<tr>
<td>II</td>
<td>Alloxan (120mg/kg ip)</td>
<td>89± 2.83</td>
<td>210±6.37</td>
<td>270± 3.40</td>
<td>274±1.78</td>
</tr>
<tr>
<td>III</td>
<td>Standard (Alloxan + Glimipride) (5mg/kg i.o)</td>
<td>92 ±1.86</td>
<td>190±13.26</td>
<td>154± 2.31</td>
<td>98± 2.90</td>
</tr>
<tr>
<td>IV</td>
<td>Test (Alloxan + A.paniculata) (400mg/kg i.o)</td>
<td>86±  2.13</td>
<td>182± 3.86</td>
<td>110±2.49</td>
<td>81± 2.26</td>
</tr>
</tbody>
</table>

Values are expressed as Mean± SEM; (n=5); Significance relative to control: ***p< 0.001

Figure -1: Effect of ethanolic extract of leaves of Andrographis paniculata in Alloxan induced rat at '0'days
**Figure 2:** Effect of ethanolic extract of leaves of *Andrographis paniculata* on Alloxan induced rat ‘3’ days

**Figure 3:** Effect of ethanolic extract of leaves of *Andrographis paniculata* on Alloxan induced rat ‘7’ days

**Figure 4:** Effect of ethanolic extract of leaves of *Andrographis paniculata* in Alloxan induced rat at ‘10’ days
IV. DISCUSSION

Nearly the kalmegh plant and their parts were discovered to be wealthy in phytochemicals, for example, terpenes, terpenoid. Terpenes are the most widely familiar terpenoids. Terpenoids containing of Andrographis paniculata has a great anti diabetic property, which is effectually comprehended in this examination. On the Other hand in this exploration we examine about the number of cycle were included a medication advancement, how potency we direct a preclinical preliminary as well as clinical preliminaries. How we acknowledge the process of isolation, separation and recognition of the medication particle, enthusiasm a dose structure and so on and portion valuation by LD50 (intense harmfulness test).[1,2]

Alloxan, one of the popular diabetogenic agent which is used to assess the antidiabetic capacity of the test compound, is a urea derivative, which is likely to inhibits glucose sensor of the Beta cell, and causes a state of insulin dependent diabetes through its ability to introduce ROS formation, as a result of which necrosis of beta cell occur. This experiment gives us the evidence that Kalmegh [Andrographis paniculata] has potent antidiabetic properties in it.

Carcinogeneces, mutagenesis, and teratogenesis, diabeticogenic, nephrotoxic, hepatotoxic, gastric ulceration, these all are caused by Alloxan. Alloxan is given by intraperitoneally, intravenously to laboratory rat in multiple sub-diabetogenic doses to produced pancreatic insulines with eventual ruin the insulin secretion in the beta cells and as a result of which diabetes mellitus occur. The frequency and brutality of lesion produced by Alloxan in pancreas, kidney GIT and Liver, gradually increased with the time of the post treatment.

Andrographis paniculata was able to successfully decrease the level of high blood sugar level to normal range. The compound succeeded to improve the uptake of glucose in alloxan induced rat. Kalmegh possess some other activities such as anti inflammatory,antispasmodic, hepatoprotective and antioxidant.

The ethanolic extract of leaves of Andrographis paniculata reduce the glucose level in Alloxan induced diabetes in rat. Hence, Andrographis paniculata extraction may real in some pathway which aggravate the diabetes mellitus. Such this experiment indicates this Andrographis paniculata extraction possibly will stimulate the secretion of insulin or the activity of insulin increase. [9, 10]

V. CONCLUSION

The present study exposes that the ethanolic extract of Andrographis paniculata significantly recover the effect of diabetes in Alloxan induced diabetic rats. Thus it concluded that the ethanolic extract of leaves of Andrographis paniculata is potential in contradiction of to diabetic.

COMPETING INTERESTS

Authors have declared that no competing interests exist

ACKNOWLEDGEMENTS

The authors are thankful to management of Bharat Technology for providing the required facilities to carry out the research work.

VI. REFERENCES


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