



**PRELIMINARY PHYTOCHEMICAL SCREENING AND IN VIVO ANTIDIABETIC
ACTIVITY OF AQUEOUS EXTRACT OF *MACROPTILIUM ATROPURPUREUM***

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ABSTRACT

Diabetes mellitus is one of the commonly occurring endocrine disorders which not only disturb the metabolism of carbohydrates, fat and proteins. It also results in reducing sensitivity of the tissue to insulin. *Macroptilium atropurpureum* is a plant which is available throughout the year in India and other countries. The present study was targeted to preliminary phytochemical analysis of *Macroptilium atropurpureum*. Based on phytochemical analysis aqueous extract was selected to evaluate antidiabetic activity in alloxan induced diabetic rats. Diabetes is induced by a single-dose intraperitoneal injection (i.p.) of alloxan (150 mg/kg) to albino wistar rats which is a toxic glucose analogue in rodents. Animals were divided into 5 groups each group consisting of 6 animals. Group IV and V was treated with aqueous extract of *Macroptilium atropurpureum* at a dose of 200mg/kg, 400mg/kg simultaneously whereas Group III was treated with glibenclamide at a dose of 10mg/kg for 10 days after induction of diabetes by alloxan. After 15 days treatment, Group V animals showed significant reduction in blood glucose levels and increase in body weight when compared to Group IV (200mg/kg) which was very well comparable with Group III (standard). Histopathological study of pancreas of diabetic rats treated with *Macroptilium atropurpureum* showed slight regeneration of beta cells. Thus in future after carrying out detailed pharmacological studies of *Macroptilium atropurpureum* it can be used as potent antidiabetic drug.

KEYWORDS: Diabetic Mellitus, *Macroptilium atropurpureum*, Petroleum ether, Chloroform, Alloxan Glibenclamide.

INTRODUCTION

As we all know diabetes is one of the major concern and long lasting illness in developing and under developed countries which causes serious health issues depending on the immunity and blood glucose levels of individuals.

Glucose acts as a fuel for all the body cells when it is present at normal level, on the other hand if glucose levels are high, it slowly erode the ability of cells in pancreas to make insulin. The organs overcompensate and insulin levels remain high on prolonged basis the pancreas is permanently damaged and also causes atherosclerosis. Initially it starts with increase urine output, excessive thirst, weight loss, aggressive hunger and in worst condition it may lead to damage of blood vessels which in turn causes renal failure, impaired vision, slow wound healing etc.

There are ample synthetic medicines which exist for diabetes with many adverse affects. But nature has

provided a supportive and safe approach for many diseases not only in worst condition but even to maintain proper health care.

India is widely distributed with many medicinal plants including more than 45,000 plant species, 10 vegetative zone and more than 15 biotics provinces.^[1]

Macroptilium atropurpureum is one of the Indian medicinal plant which belongs to the family fabaceae. In India, it is found in moist tropical and sub tropical areas, best yield is during summer and early autumn and also available in tropical and subtropical regions of North, Central and South America, as far north as Texas in the USA and as far south as Peru and Brazil.^[2]

In our present study plant based diabetic activity has been chosen in order to avoid synthetic medicines which possess many adverse effects.

MATERIALS AND METHODS

Plant: The plant of *Macroptilium atropurpureum* was obtained from Agricultural University (Hyderabad). The plant specimen was identified and authenticated by L. Rasingam, Scientist In-charge, Botanical Survey of India, Hyderabad. A voucher specimen no. BSI/DRC/2017-18/Tech/935. Plant was collected and shade dried, then powdered in a mechanical grinder and passed through a sieve no.40 to obtain powder of desired particle size. The powder was stored in an airtight container and kept in dry place.

PREPARATION OF EXTRACT^[3]

Preparation of Petroleum Ether Extract

The dried powder material (750gms) was subjected to soxhlet extraction with petroleum ether. The marc was pressed after the extraction and the filters were pooled and concentrated under reduced pressure to obtained dried solid mass.

Preparation of Aqueous Extract

1. About 400gms of dried marc was taken in a 1000 ml of beaker and macerated with 500ml of distilled water to which 10ml of chloroform was added as a preservative and kept it for seven days with occasional shaking daily in a closed vessel.

2. The supernatant was decanted and the marc was pressed then the pooled extract was concentrated on water bath at 50^o c to get a dry solid mass.

Preliminary phytochemical studies^[4]

Both the extracts were subjected to preliminary phytochemical screening by following standard methods for detection of active constituents such as Carbohydrates, Proteins, Glycosides, Alkaloids, Tannins, Saponins, Flavonoids, Phenols, Amino acids, Steroids, Vitamins, Terpenoids, Fats and oils.

Screening of acute oral toxicity study of drug samples in Mice (OECD 423)^[5]

The acute oral toxicity studies were carried out using the limit test procedure according to OECD Test Guidelines 423 using Mice.

Animals: Albino male wistar rats weighing 120–160g were used for this experiment. The rats were housed in a group of six in polypropylene cages at controlled room temperature 25°C ± 2°C and relative humidity 55% and 12 h light-dark cycle. They were fed *ad. Libitum* during the experiment.^[6]

RESULTS AND DISCUSSION

Percentage of yield

Table 1: Percentage Yield of Extract

| S.no | Extract | Nature of extract | Colour | Weight (GM) | Percentage yield (%) |
|------|-----------------|-------------------|------------|-------------|----------------------|
| 01 | Aqueous | SEMI-SOLID | Dark brown | 46 | 4.6% |
| 02 | Petroleum ether | SEMI-SOLID | Dark brown | 17 | 1.7% |

Induction of diabetes: Food was withdrawn 12 h prior to the induction of diabetes. Thereafter, they were injected with Alloxan (150 mg/kg/day, i.p.) dissolved in normal saline for five days. The blood glucose levels was checked before and after administration of alloxan to confirm the diabetes, Rats with blood glucose levels higher than 240 mg/dl were considered diabetic and further used for *in vivo* studies.^[7]

The animals were randomly divided into five groups (N = 6/groups).^[8]

Group 1: Normal control Rats

Group 2: Negative control Rats (only alloxan) 150mg/kg/day for 5 days (by i.p route)

Group 3: Standard (Glibenclamide) 10mg/kg (by oral route)

Group 4: 200mg/kg of aqueous extract of *Macroptilium atropurpureum* (by oral route)

Group 5: 400mg/kg of aqueous extract of *Macroptilium atropurpureum* (by oral route)

EVALUATION OF ANTI-DIABETIC ACTIVITY^[9]

The animals were housed individually and maintained on normal food and water *ad. Libitum*. Animals were periodically weighted before and after experiments. Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study.

Blood glucose levels were determined by using simple glucometer equipment, when the blood is withdrawn by retro-orbital puncture method a single drop is enough to determine the blood glucose levels. A drop of blood was used on glucometer strip which immediately gives the blood glucose readings.

Blood sample was collected for monitoring bleed glucose levels on first, fourth, seventh and tenth day of the treatment. With the help of Accu-check glucometer which is known to be very efficient and accurate in giving quick results.

Histopathological Studies: On the 15th day, all the animals were sacrificed and pancreases were removed. Pancreatic samples were taken for histopathology and the tissue samples were processed in tissue processor (Leica made). Pancreatic sections were stained with Haematoxylin and Eosin Y (H/E) dyes. The sections of pancreas were observed under light microscope (Olympus) for histopathological study.^[10]

Table 2: Preliminary phytochemical screening.

| S.no | Name of the Secondary metabolite | Petroleum ether extract | Aqueous extract |
|------|----------------------------------|-------------------------|-----------------|
| 1 | Carbohydrates | + | + |
| 2 | Proteins | + | + |
| 3 | Glycosides | - | + |
| 4 | Alkaloids | - | - |
| 5 | Tannins | - | - |
| 6 | Saponins | - | + |
| 7 | Flavonoids | - | ++ |
| 8 | Phenols | + | ++ |
| 9 | Amino acids | - | - |
| 10 | Steroids | - | ++ |
| 11 | Vitamins | - | - |
| 12 | Terpenoids | + | ++ |
| 13 | Fats and oils | + | - |

++ = High, + = Good, - = Absent

Table 3: Acute Oral Toxicity Studies

| S. No. | Response | Concentration of Plant Extract (2000 mg/kg) |
|--------|-------------------|---|
| 1 | Motor Activity | N |
| 2 | Grooming | Ab |
| 3 | Touch Response | N |
| 4 | Pain Response | N |
| 5 | Tremors | Ab |
| 6 | Convulsions | Ab |
| 7 | Lighting Reflex | N |
| 8 | Gripping Strength | N |
| 9 | Pinna Reflex | P |
| 10 | Corneal Reflex | P |
| 11 | Writhing | N |
| 12 | Pupils | N |
| 13 | Urinations | N |
| 14 | Salivation | N |
| 15 | Skin Color | N |
| 16 | Lacrimation | N |
| 17 | Defecation | N |
| 18 | Diarrhea | Ab |
| 19 | Coma | Ab |

N = Normal, P = Present, Ab = Absent

No sign of toxicity and mortality in the female mice were observed even after receiving a maximum dose 2000mg/kg aqueous extract of *Macroptilium*

Preliminary phytochemical study revealed the presence of Carbohydrates, Proteins, Phenols, Terpenoids, Fats and oils and absence of Glycosides, Alkaloids, Tannins, Saponins, Flavonoids, Amino acids, Steroids, Vitamins in petroleum ether extract of *Macroptilium atropurpureum*. Whereas, it revealed the presence of Carbohydrates, Proteins, Glycosides, Saponins, Flavonoids, Phenols, Steroids, Terpenoids and absence of Alkaloids, Tannins, Amino acids, Vitamins, Fats and oils, in aqueous extract of *Macroptilium atropurpureum*.

atropurpureum. Therefore, 1/5th and 1/10th of the maximum tolerated dose was selected in further *in vivo* studies.

ANTI-DIABETIC

Table 4: Effect of *Macroptilium atropurpureum* extract on fasting blood glucose Level of diabetic rats.

| Groups | Day 1 | Day 4 | Day 7 | Day 10 |
|-----------|--------------|-------------|-------------|--------------|
| Group I | 92.30 ± 1.29 | 90.8 ± 0.8 | 95 ± 1.3 | 91 ± 0.7 |
| Group II | 255.1 ± 3.8 | 257.7 ± 3.1 | 263.3 ± 3.4 | 267 ± 2.6 |
| Group III | 234.2 ± 3.9 | 201.5 ± 3.9 | 170.3 ± 4.1 | 140 ± 3.2 |
| Group IV | 260.8 ± 3.2 | 238.3 ± 2.9 | 195.8 ± 1.7 | 157.5 ± 2.1 |
| Group V | 243 ± 3.1 | 216 ± 2.9 | 176.7 ± 3.1 | 148.7 ± 2.67 |

There was significant elevation in fasting blood glucose level of group II induced diabetic rats compared to group I. Treatment of diabetic rats with *Macroptilium atropurpureum* extract for 15 days caused marked reduction in fasting blood glucose level compared to

group II. Group V animals results was very well comparable with group III treated diabetic rats which showed marked reduction in fasting blood glucose level.

BODY WEIGHT

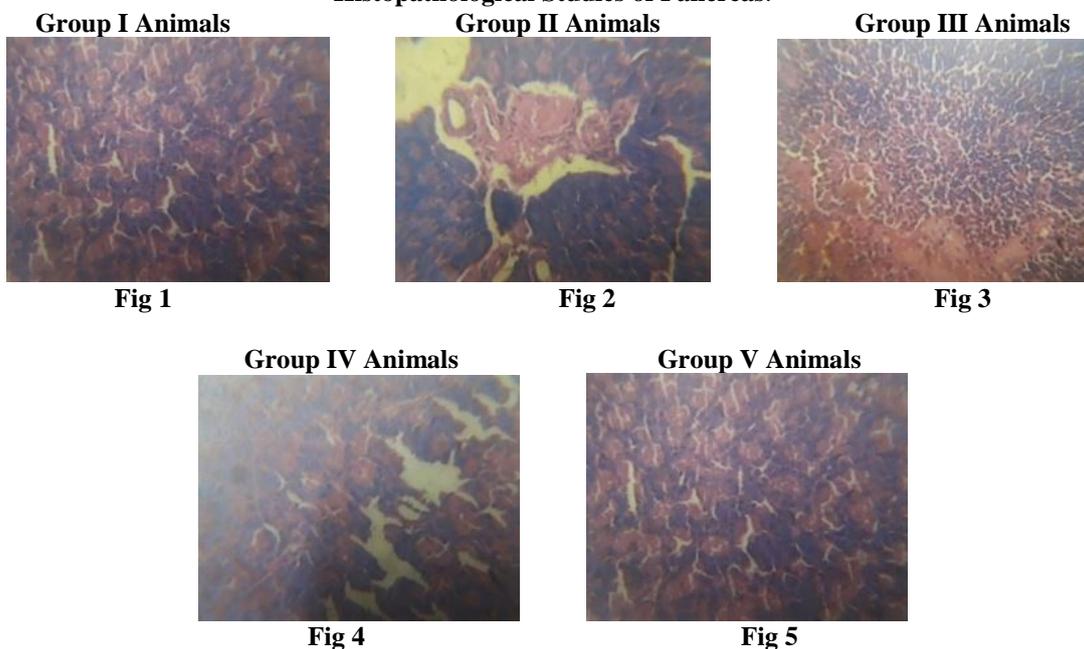
Table 5: Effect of *Macroptilium atropurpureum* Extract on Body Weight of Diabetic Rats

| Groups | Day 1 | Day 4 | Day 7 | Day 10 |
|-----------|-------------|---------------|---------------|---------------|
| Group I | 138 ± 19.00 | 141.2 ± 19.00 | 145.6 ± 16.72 | 150.3 ± 13.49 |
| Group II | 147 ± 08.17 | 151.2 ± 13.49 | 151.5 ± 19.24 | 158.3 ± 16.11 |
| Group III | 147 ± 13.05 | 142.3 ± 13.40 | 145.2 ± 16.32 | 143.3 ± 10.94 |
| Group IV | 147 ± 17.16 | 144.8 ± 17.43 | 149.7 ± 18.16 | 157.2 ± 20.62 |
| Group V | 147 ± 16.53 | 147.8 ± 13.37 | 147.2 ± 13.63 | 146.2 ± 15.01 |

There was significant increase in the body weight of Group II animals compared to Group I animals. Group V

was very well comparable with group III treated diabetic rats which showed marked improvement in body weight.

Histopathological Studies of Pancreas:



Histopathology of the pancreas of Group I animals showed islets cells with normal acini and abundant of cytoplasm, as shown in Figure 1. Pancreas of Group II animals showed atrophy with degeneration and necrosis of pancreatic tissue and invasion of connective tissues in the parenchyma of pancreatic islets, as shown in Figure 2. The pancreas of Group III animals revealed partial regeneration of islet cells with presence of numerous beta cells in pancreatic islets, as shown in Figure 3. The pancreas of *Macroptilium atropurpureum* Linn. extract Group IV & V showed slight regeneration of pancreatic cells with normal islet cells, as shown in Figures 4 and 5.

CONCLUSION

The results of the study indicate that *Macroptilium atropurpureum* has got potential to reduce the blood glucose levels within a short period of time and also it has potential to improve the glucose tolerance after a treatment period of 15 days. In conclusion our study

suggests that *Macroptilium atropurpureum* may have beneficial effect in diabetes mellitus and may improve glucose tolerance as well. Thus it holds the scope of a new generation of antidiabetic drug. However, there is need for further studies on experimental animals and human beings, using various active principles, to establish its usefulness, exact mode of action and toxicity data.

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