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# Antidiabetic Activity of Polyherbal Extract on Alloxan Induced Diabetic Rats

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**Abstract:** Polyherbal extract which consists of ethanolic extract of *MadhucaLatifolia, AristolochiaBracteata, AristolochiaIndica* was investigated for its possible antidiabetic effect in alloxan-induced diabetic rats and to assess the synergistic activity of three plants. The animals were grouped into four six animals each. The first group received normal saline solution(0.9% sodium chloride), which was used as a negative control. The second group was used as a positive control. The third group received 200mg/kg bodyweight of polyherbal extract orally. The fourth group received 10mg/kg bodyweight of standard drug Glibenclamide orally. Diabetic rats treated with polyherbal extract at a dose of 200 mg/kg showed significant decrease in blood glucose level at 12th and 24thhour from initial levels. A significant time dependent antidiabetic effect was shown throughout the period of study. The antidiabetic effect of polyherbal extract was nearly comparable than that observed with glibenclamide.

**Key words:** Polyherbal Extract · Antidiabetic · *Madhuca latifolia* · *Aristolochia bracteata* · *Aristolochia indica* · Glibenclimide

## INTRODUCTION

Humans have relied on the nature for their basic needs for food, shelter and clothing throughout the ages [1]. For thousands of years, Plants have paved path for the traditional medicine systems and continue to provide mankind with new remedies [2]. World health organization has estimated that several plants are known to have various medicinal applications in various cultures [3] and WHO also estimated that about 4 billion people, 80% of the world population presently use herbal medicine in order to have a primary health care. Morethan 50% of all the drugs in clinical use in the world today have been represented by the natural products and their derivatives and higher plants contribute not less than 25% [4].

The incidence of diabetes is growing rapidly worldwide. For example, it is estimated that more than 180 million people worldwide are afflicted with diabetes and its prevalence is expected to be more than double by the year 2030. About 5% of the global populations affected by diabetes and the management of diabetes without any side effects is still a challenge to the medical system [5-7]. Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin [8]. Diabetes mellitus(DM) is not a single disease, it consists of a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular disease. These metabolic abnormalities result, in part, from a deficiency of the blood sugar-lowering hormone insulin. The disturbances in the level of insulin lead to increase in glucose levels in blood, resulting in diabetes mellitus. According to Expert Committee on the Diagnosis and Treatment of Diabetes Mellitus, 2003, most patients can be classified clinically as having either type 1 or type 2 DM. Type1 diabetes (formerly insulin-dependent diabetes mellitus-IDDM-or juvenile-onset diabetes) and Type 2 diabetes (formerly non-insulin-dependent diabetes mellitus-NIDDM-or maturity-onset diabetes). which is a multifactorial disease [9]

People with diabetic ailments, commonly complain symptoms like frequent urination, extreme thirst and hunger, fatigue or unusual tiredness, weight loss etc [10].

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*Aristolochiaindica* which belongs to the family aristolochiacae is distributed throughout all provinces of India, Srilanka, Nepal, Bangladesh [11]. The medicinally active parts of the plant are the Root, rhizome, leaves although the exact mechanism is unknown [12]. Plant posses emmenagogue, abortifacient, diuretic and antibilious properties, anticancer and antioestrogenic activities [16]. The preliminary investigations carried out on the plant to identify various chemical constituents and found to have nitrophenanthrene compound aristolochicacid [16].

*Aristolochiabracteata*which belongs to the family Aristolochiaceae found in upper gangetic plain, west Bengal, Gujarat and peninsular India [12,13]. The Whole plant is medicinally active although the exact mechanism is unknown [12]. The plant is said to have oxytocic, anthelmintic, cathartic properties [16] Through preliminary investigations it has been identified that the plant constitutes alkaloid magnoflorine, aristolochic acid and fatty oil, ceryl alcohol, beta-sitosterol and aristolochic acid [16].

*Madhucalatifolia* which belongs to the family Sapotaeceae [14, 15]. The medicinally active parts of the plant are the Flowers, Fruit, Bark, although the exact mechanism is unknown [12]. The active principles confirm cooling, tonic, demulcent properties, spermicidal activity and anti-inflammatory activity [16]. The plant contains active principles like vitamins(Carotene, Pantothenicacid, Biotin, Inositol, Ascorbic acid, Thiamine, Riboflavin, Niacin, Folic acid), anthrocyanins, betains and salts of malic and succinic acids, enzymes (Catalase, oxidase, invertase, maltase, amylase and emulsin), terpenoids including alpha, beta amyrin acetate, 3-beta mono caprylic ester of erythrodion, 3-beta capryloxyoleanolic acid and an acetate. In addition n-hexacosanol, beta-D-glucoside of beta-sitosterol and free beta-sitosterol, lupeol acetate and alpha-spinasterol, beta-carotene, sitosterol its glucoside, palmitic acid, monopalmitate, oleanolic acid, quercetin were identified [16].

The antidiabetic activity of Aristolochiaindica, Madhucalatifolia has already been proven. Some other species of aristolochia like Aristolochia bracteata may also have antidiabetic activity which has not been proved. The present study is to investigate and rationalize the antidiabetic activity of polyherbal extract of Aristolochiaindica, Aristilochiabracteata, Madhu calatifolia and to assess the synergistic activity of these three plants.

## MATERIALS AND METHODS

**Plant Collection and Processing:** The plants were collected from the Regional Forest Research Centre (RFRC), Rajahmundry and were authenticated by RFRC and Pharmacognosy and Phytochemistry department, GIET School of Pharmacy. The collected plant material was cleaned and placed in a clean tray and allowed for shade drying. The dried material was powdered coarsely in grinder mill. The powder passed through 40 # mesh size and stored in an airtight container at room temperature in order to avoid contact with moisture and air.

**Preparation of Extract:** Coarse powder of around 100g was weighed and bag containing powder was packed in soxhlet apparatus. Extraction was done using ethanol as solvent [12]. Appearance of colourless solution in the siphon tube of the soxhlet apparatus was taken as the endpoint of extraction. The extract was concentrated to 3/4th of its original volume by distillation and this extract was made into a thick paste by concentrating it on the thermostat controlled water bath. The collected extract was stored at 40°C for further use. The alcoholic extract obtained was used for to evaluate the antidiabetic activity and to assess synergistic activity of the three plants.

**Animals:** Healthy young albino rats of wistar strain of either sex weighing 180-200gm procured from the animal house of GIET School of Pharmacy, Rajahmundry, were used for the study. These rats were housed in standard environmental conditions of temperature 22±10°C and relative humidity 60 ± 5%.They were maintained on 12h dark cycle and 12hr light cycle with free access to a standard commercial diet and water. The animals were deprived of food but not water for 18 hrs before experimentation. Experiment was performed according to the guidelines and the study was approved by the Institutional Animal Ethics Committee (IAEC-CPCSEA Registration No-1069/AC/07/CPCSEA).

**Experimental Induction of Diabetes [18]:** The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 120mg/kg body weight. Alloxan is capable of producing fatal hypoglycaemia as a result massive pancreative insulin release and therefore the rats were treated with 20% glucose solution (15-20 ml) orally after 6 hr[19]. After 2

weeks of the treatment, rats with severe hyperglycemia i.e. with a blood glucose level of more than 400mg/dl were used for the experiment.

**Experimental Design [18]:** In this experiment, diabetes was induced two weeks before starting the experiment in the rats. The rats were divided into four groups after the induction of alloxan diabetes and six rats were used in each group. The first group received normal control (vehicle only). The second group served as diabetic control. The third group received200mg/kg bodyweight of ethanolic polyherbal extract orally and the fourth group received glibenclimide(10mg/kg bodyweight) orally.

**Determination of Blood Glucose Level:** Blood glucose was measured at 1, 3, 6, 12 and 24 hr after single oral administration. Blood was withdrawn from the tail vein each time. The blood glucose level was determined by glucose oxidase peroxidase method [15] by using biochemical analyzer inkarp –vital eon one 00183, made in Italy.

**Statistical Analysis [18]:** The quantitative measurements in all the experiments were made on 6 animals in each group and the values were expressed as mean ± SEM (Standard error mean). The data were subjected to one way ANOVA to determine the significance of changes to analyze the significance of difference within the experimental groups. P values with <0.05 were considered as statisticaly significant.

## RESULTS AND DISCUSSIONS

In the present study, the antidiabetic activity of polyherbal extract of *MadhucaLatifolia, Aristolochia Bracteata, AristolochiaIndica* was evaluated in normal and Alloxan induced diabetic rats. Table 1 and Fig 1 showed the effect of polyherbal extract on blood glucose

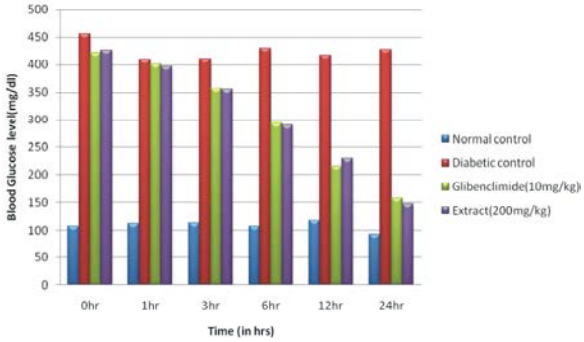


Fig. 1: Graph representing the effect of polyherbal extract on alloxan induced diabetic ratsApril 20, 2013

level in Alloxan induced diabetic rats. Diabetic rats were treated with polyherbal extract at a dose of 200 mg/kg showed significant decrease in blood glucose level at 12th and 24th hour from initial levels. A significant time dependent antidiabetic effect was seen throughout the period of study. The present study indicated that the polyherbal extract of *Madhuca Latifolia, Aristolochia Bracteata, AristolochiaIndica* possess antidiabetic properties which suggests the presence of biologically active components. Alloxan causes diabetes through its ability to destroy insulin producing beta cells of pancreas [21,22]. In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells leading to induction of necrosis [23,24]. Cytotoxic action of alloxan is mediated by reactive oxygen with simultaneous massive increase in cytosolic calcium concentration leading to rapid destruction of beta cells [25]. Maintenance of blood glucose level with polyherbal extract treated rat indicates the effectiveness in experimental diabetic animal as shown in Table 1. The significant decrease of blood glucose was found in 12thand 24th hr than that of the initial. Experimental studies reveals that alcoholic extract from polyherbal extract orally administered, produced a significant decrease in blood glucose level in alloxan induced diabetes in rats.

Table 1: Effect of polyherbal ethanolic extract on blood glucose levels of alloxan induced diabetic rats (mg/dl)

| Animal groups | Dose | 0 hr | 1hr | 3hr | 6hr | 12 hr | 24 hr |
|---|---|---|---|---|---|---|---|
| Normal | Water | 108± 6.8 | 112±3.6 | 113±7.1 | 108±5.9 | 118±3.2 | 101 ±5.7 |
| Diabetic control | Alloxan (200mg/kg) | 456±20.8 | 409±3.1 | 410± 8.2 | 430±50.2 | 417±73.1 | 427±6.2 |
| Standard | Alloxan (200mg/kg)+Glibenclamide (120mg/kg) | 422± 32.4 | 401±53.1 | 357±22.2* | 293±18.2* | 216±24.2* | 158±22** |
| Test | Alloxan(200mg/kg)+Polyherbal extract (100mg/kg) | 426± 7.3 | 397± 26.2 | 356± 9.6* | 290± 7.5* | 229± 8.6* | 147±28.2** |

Data are presented as Mean±SEM.*p<0.01 and **p<0.001 when compared to Control

## CONCLUSION

From the above study it was concluded that the ethanolic polyherbal extract of *Madhuca Latifolia, Aristolochia Bracteata, AristolochiaIndica*possess potential activity in decreasing the blood glucose level and the three plants have synergistic activity in reducing the blood glucose level. Further research is needed to isolate lead compound from this polyherbal extract to find the exact active constituent and mechanism of action responsible for the antidiabetic activity. Being a herbal extract it may gain its own benefits as therapeutic agent in diabetes research.

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