

Hypoglycemic activity of *Aegeles mermoles* in alloxan induced diabetic mice

¹ Vedhpal jeyamani S*, ² Ramya N, ¹ Roosewelt C, ³ Murugan M and ¹ Gunasekeran V.

¹ Jaya college of paramedical sciences, Thiruninruvur, Chennai, Tamilnadu, India.

² K K college of pharmacy, Gerugambakkam, Chennai, Tamilnadu, India.

³ EGS Pillay college of pharmacy, Karaikkal, Tamilnadu, India.

*Corresponding Author: E-Mail: swetha21112000@gmail.com

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ABSTRACT

Ethanollic bark extract of *Aegeles mermoles* was carried out using soxhlet apparatus. The extract was evaluated for preliminary phytochemical studies, acute toxicity studies and hypoglycemic activity. Acute toxicity study revealed that the ethanollic bark extract even at high dose (2000mg/ kg) produced no death and found to be safe. The hypoglycemic activity was evaluated in normal and alloxan induced diabetic mice. The chloroform fraction of the bark extract was found to possess significant hypoglycemic activity when compared to standard Glibenclamide.

Keywords: *Aegeles mermoles*, hypoglycemic activity, Diabetes mellitus.

1. INTRODUCTION

Diabetes is a metabolic disorder associated with many other metabolic functional alterations. The number of people in the world with diabetes has increased dramatically over recent years. Indeed, by 2020 it has been estimated that the diabetic population will increase to 221 million around the world. There is a lack of exact etiology and specific drugs for correcting all abnormalities in the treatment of diabetes [1,2]. The main objective of the study was to assess the anti diabetic potential of leaves of *Aegeles mermoles*.

2. MATERIALS AND METHODS

2.1. Extraction

The leaves were dried under shade, crushed into coarse powder. The powder was loaded in to the Soxhlet extractor in 5 batches and was subjected to extraction for about 48 hours with ethyl alcohol (70%). After extraction the solvent was distilled off and extract was concentrated on water bath to a dry residue.

2.2. Fractionation

The concentrated 70% ethanollic extract was subjected to successive fractionation with toluene, chloroform, ethylacetate and n-butanol. The fractions were concentrated and dried over anhydrous sodium sulphite (Na₂SO₃) and concentrated to small volume and then

evaporated to dryness. All the fractions were kept in a desiccator and stored in refrigerator for phytochemical investigation and pharmacological studies [3-6].

2.3. Preliminary phytochemical studies

The ethanollic extract and individual fractions were subjected to qualitative chemical investigations for the identification of the phytoconstituents viz., glycosides, carbohydrates and alkaloids.

2.4. Pharmacological studies

2.4.1. Acute toxicity studies

Albino mice of either sex weighing 20-25 gms were used to determine LD₅₀ of various fractions. The gum acacia 2% was used as a vehicle to suspend various fractions were found to be safe upto the higher dose level of 2000mg/kg of body weight.

2.4.2. Animals

In bred wistar albino mice weighing 20-25 gms were housed in large spacious hygienic polypropylene cages and animals had 12+1 hour day and night cycle. The animals were allowed to standard pellet diet and tap water ad libitum. The husk for the purpose of keeping as a bed to the animals was cleaned and autoclaved. Before the animals were kept in the polypropylene cages were sterilised along with water feeding bottles.

2.4.3. Preparation of diabetic mice

Experimental diabetes in mice was induced by injecting alloxan monohydrate intraperitoneally at a dose of 120mg/kg body weight in Ice cold citrate buffer pH 4.5. After 72 hours of blood was collected from the tail vein under mild ether anaesthesia of all surviving mice and blood glucose levels were determined colorimetrically by using autoanalyzer microlab 200. Mice with blood sugar levels of 200-350 mg/dl were considered as diabetic and were employed in the study.

The mice were randomly divided into 6 groups of six animals each. Group I served as Diabetic control and received 0.3% CMC orally. Group II served as positive control and received glibenclamide (10 mg/Kg). Group III + IV received suspensions of fractions, orally at a dose of 150mg/kg. The treatment was continued for 8 days by administering the respective fractions of the drug or 0.3% CMC, twice daily [6-8].

2.4.4. Collection and Processing of Blood for Estimation of glucose and Biochemical parameters

On the ninth day of the therapy, blood samples were collected from the tail vein under

mild ether anaesthesia in Eppendroff's tubes containing anticoagulant plasma was separated by centrifuging the samples at 500 rpm for 10 mins and stored in a refrigerator until analyzed.

2.5. Statistical analysis

Data were expressed as Mean \pm SE and analyzed statistically using one way ANOVA by Dunnett's multiple comparison test. The results were analysed statistically and compared with those of control using students -t test. The level of significance was fixed at $P < 0.05$.

3. RESULTS

3.1. Phytochemical analysis

The ethanolic extract of *Aegeles mermales* showed presence of alkaloids, carbohydrates and glycosides.

3.2. Effect on blood glucose

Alloxan (120 mg/kg, i.p) elevated the blood glucose levels along with significant decrease in the body weight of the mice ($p < 0.02$ decrease) which was partially restored or improved upon administration of fraction significantly decreased ($p < 0.01$) the elevated glucose level in comparison to the non treated diabetic mice.

Table - 1: Effect of various Fractions of *Aegeles mermales* on body weight and blood glucose level in Alloxan induced Diabetic mice. Data are expressed as mean \pm SE (n=6)

Group	Body Weight(g)		Glucose level (mg/dl)		
	Before	After	Normal	After Alloxan	After drug treatment
Solvent Control	190.6 \pm 13.7	151.6 \pm 6.9	77.6 \pm 8.9	270.3 \pm 32.3	313.6 \pm 31.2
Positive Control	181.7 \pm 19.2	169.6 \pm 9.2	86.0 \pm 4.9	287.6 \pm 31.1	143.6 \pm 34.4
Toluene Fraction	200.3 \pm 6.8	177.7 \pm 10.4	85.6 \pm 5.8	288.0 \pm 17.0	270.3 \pm 15.6
Chloroform Fraction	182.7 \pm 9.3	169.0 \pm 9.8	79.3 \pm 8.1	373.0 \pm 34.9	226.3 \pm 36.7
Ethyl Acetate Fraction	191.6 \pm 11.8	160.3 \pm 6.8	80.0 \pm 5.1	286.0 \pm 18.8	264.3 \pm 15.5
n-Butanol Fraction	183.0 \pm 17.4	159.3 \pm 7.8	78.0 \pm 8.6	315.6 \pm 21.3	263.1 \pm 28.3

"+" denotes increase and "-" denotes decrease in Hypoglycemic activity.

4. DISCUSSION

The average percentage yield of ethanolic (70%) extract of leaves of *Aegeles mermales* was found to be 15.80% w/w and the corresponding value for toluene, chloroform, ethylacetate and n-butanol fractions. Carbohydrates, glycosides and alkaloids were found to be present in ethanolic extract and in various fractions.

Alloxan has been shown to produce hyperglycaemia which seemed to retain partial beta cell activity and ineffectiveness in severe diabetes may be due to complete destruction of Beta cells.

Insulin deficiency leads to various metabolic alterations in the animals viz., increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases. Several investigators demonstrated that glycosides, alkaloids, flavonoids and steroidal compounds as active ingredients in hypoglycemic plants. Thus the hypoglycemic effect produced by the fraction may be due to alkaloids and flavonoids present in the fraction.

From this study, we can state that the tested fraction has beneficial effects on blood glucose levels as well as body weight. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of action.

5. REFERENCES

1. Yohanarasimhan, S.N., Medicinal Plants of India, Vol -II, 2000; 431-432.
2. Dhanabal S.P, Kokate C.K, Ramanathan M, Elango K, Kumar E P, Subburaj T, Manimaran S and Suresh B. Antihyperglycemic activity of Polygala arvensis in alloxan induced diabetic rats, **Indian drugs**, 2004; 41: 690- 695.
3. Jayaka B, Suresh B, Antihyperglycemic and hypoglycemic effect of Aporosa lindleyana in normal and alloxan induced diabetic rats. **Journal of Ethnopharmacology**, 2003; 84: 247-249.
4. Geeta Watal and Achyut Narayanan Kesari. Hypoglycemic and antihyperglycemic activity of Aegeles marmelos seed extract in normal and alloxan induced diabetic rats. **Journal of Ethnopharmacology**, 2006; 107: 374-379.
5. Shanmugasundaram KR, Panneerselvam SP and Shanmugasundaram RB. Enzyme changes and glucose utilization in diabetic rabbit: The effect of Gymnema sylvestrae R. **Br. J. Ethnopharmacol.**, 1983; 7: 205-216.
6. Begum N and Shanmugasudnaram KR. Tissue phosphates in experimental diabetes. **Arogya: J. Health Sci.**, 1978; 4: 129-139.
7. Felig P, Marliss E, Ohman J and Cahill Jr J F. Plasma amino acids level in diabetic ketoacidosis. **Diabetes**. 1970; 19: 727.
8. Oliver B and Oval. Oral Hypoglycaemic plants in West Africa: **J. Ethnopharmacol**, 1980; 2: 119-128.
9. Sheeja C and Augusti KT. Insulin spring action of leucopenargonin derivative isolated from Ficus bengalensis linn India. **J of Exp Biol**, 1995; 33: 608-611.