Effect of *Alangium salvifolium* (Alangiaceae) on Dexamethasone Induced Insulin Resistance in Rats

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**ABSTRACT**

The present study was undertaken to investigate the antihyperglycemic and antihyperlipidemic effects of methanol extract of *Alangium salvifolium* (ASME) on low dose dexamethasone induced insulin resistance in rats. Male wistar rats weighing 250-300 g were divided into different groups. Dexamethasone was administered by injecting subcutaneously 2μg/day in divided doses for 28 days. ASME (200 mg & 400mg/kg/day/orally) was co-administered for 28 days. At the end of experimental period, blood samples were collected by retro-orbital plexus puncture for estimation of plasma glucose, insulin, triglycerides, total cholesterol & TGTT was carried out. Dexamethasone treatment for 28 days lead to significant increase in fasting plasma glucose, plasma insulin and triglyceride level, total AUC of plasma glucose during OGTT, HOMA-IR index, when compared to control. Co-administration with different doses of ASME significantly prevented the dexamethasone induced increase in all biochemical parameters. Studies clearly demonstrated that methanolic extract of *Alangium salvifolium* leaves possess antihyperglycemic and antihyperlipidemic effects which may be due to the antioxidant and insulinotropic effect of extract.

**Key words:** Insulin resistance, Antioxidant, Dexamethasone, *Alangium salvifolium*.

**INTRODUCTION**

Insulin resistance (IR) is a common pathologic state in which target cells fail to respond to ordinary levels of circulating insulin. It results in inability of insulin to provide normal glucose and lipid homeostasis. Hence, higher than normal concentrations of insulin are needed in order to maintain normoglycaemia. Insulin resistance is also a feature of a number of health disorders, including obesity, glucose intolerance, dyslipidemia and hypertension clustering in the so-called metabolic syndrome (also commonly referred to as syndrome X). Insulin resistance has elicited great interest in medical and scientific communities because of its association with cardiovascular disease.

Glucocorticoids a widely used anti-inflammatory and immunosuppressive agent, is associated with several adverse effects such as impaired insulin sensitivity and hypertension. Dexamethasone is a widely used member of glucocorticoids has been shown to reduce cellular glucose uptake affecting the glucose transport system per se, with no direct effect on the insulin receptor. Thus dexamethasone alter glucose metabolism and in turn they have a role in the development of peripheral insulin resistance.

Increased evidences from clinical and experimental studies suggest that oxidative stress plays a major role in pathogenesis of diabetes mellitus. Abnormally high levels of free radicals and simultaneous decline in antioxidant defense mechanism may cause tissue damage and develop insulin resistance, although, glucose, proteins and lipids are among the prime targets. Hence, recently researcher suggested a different and innovative approach to a possible "causal" antioxidant therapy, eg. the flavonoids. Many flavonoid containing plants serve as a hidden wealth of potentially useful natural products for diabetes control. Nonetheless, few plants have received scientific or medical scrutiny, despite recommendations by the World Health Organization in 1980.

*Alangium salvifolium* (Alangiaceae) is a deciduous, rambling shrub of tree, uo 10 m in height with a maximum girth of 1.2 m, armed or unarmed grows wild in the plains and mid-hills of India. The literature survey reveals that the plant *Alangium salvifolium* claimed to Antidiarrhoeal, Anti-leprotic, Antipyretic, anthelmintic, Hypoglycaemic. Acidic and astringent, laxative, tonic, haemorrhagic and contains phytoconstituents like Alkaloids, Steroids and triterpenoids. Leaves of *Alangium salvifolium* claimed to have hypoglycaemic action, but no scientific report is yet available. Hence present study has been planned to evaluate the antihyperglycemic effects of *Alangium salvifolium* leaves extract on dexamethasone induced insulin resistance in rats.

**2. MATERIALS AND METHODS**

2.1 Materials: Dexamethasone was procured from Zydus cadila Ltd. Ahmedabad. Radio Immuno Assay Kit for Insulin- BI-INSULIN IRMA was procured from Cisbio, France. Glucose, Cholesterol, and Triglycerides kits were procured from ERBA diagnostic Mannheim Gmbh Germany.

2.1.1 Collection of plant materials: Fresh leaves of *Alangium salvifolium* were collected from the botanical garden of S.K. Arts and H.S.K. Science College, Vidyanagar, Hubli, Karnataka, in the month of June. The leaves of *Alangium salvifolium* were cleaned, shade-dried for 30 days at room temperature, crushed to a coarse powder and preserved for further processing.

2.1.2 Preparation of methanol extract of *Alangium salvifolium* leaves: The coarsely powdered form of shade-dried leaves was subjected for defatting with pet. ether using Soxhlet apparatus. Coarsely powdered defatted leaves were then extracted with methanol. The solvent was recovered using rotavapour.

2.2 Preparation of melatonin extract of *Alangium salvifolium* leaves: The acutely powdered form of shade-dried leaves was subjected for defatting with pet. ether using Soxhlet apparatus. Coarsely powdered defatted leaves were then extracted with methanol. The solvent was recovered using rotavapour.

2.2.1 Acute oral toxicity in mice: The acute toxicity tests for melatonin extract of *Alangium salvifolium* leaves were performed on albino mice weighing between 25-30 gm. The step-wise Up and Down method was adopted for toxicity studies. The animals were starved overnight and randomly divided into six groups (n=3). Methanol extract suspensions in dose levels of 100, 500, 1000, 2000, 3000 and 5000 mg/kg were administered intragastrically. The animals were noted for compound related toxicity signs and mortality individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours.
No mortality and no signs of toxicity were found even after administration of a limit dose of 2000 mg/kg body weight of extract; hence 1/10 of the dose was taken as effective dose (200 mg/kg body weight) for the extract as defined by fixed LD<sub>50</sub>, cut off values. Two doses 200 mg/kg and 400 mg/kg body weight were selected for the present study.

### 2.3 Animals & experimental study design:

All the experimental procedures were carried out in accordance with committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines. All the experimental procedures were approved by the institutional animal ethical committee (IAEC).

Male wistar rats weighing 250-300g were procured and housed in the environmentally controlled room with 12-h light/dark cycle and had free access to food and water. After seven days of acclimatization period, they were randomly selected for different experimental groups each containing seven rats as follows. I. Control: Injected with saline subcutaneously and serve as normal control. II. Dexamethasone: Injected with Dexamethasone 2µg/day subcutaneously for 28 days, in divided dose 1µg at 8:00 AM and 1µg at 8:00 PM daily. III. Dexamethasone + ASME 400 mg/kg: Injected with Dexamethasone 2µg/day subcutaneously for 28 days, in divided dose 1µg at 8:00AM and 1µg at 8:00PM daily and treated with ASME 400 mg/kg orally in a volume of (5 ml/kg) for 28 days. IV. Dexamethasone + ASME 400 mg/kg: Injected with Dexamethasone 2µg/day subcutaneously for 28 days, in divided dose 1µg at 8:00AM and 1µg at 8:00PM daily and treated with ASME 400 mg/kg orally in a volume of (5 ml/kg) for 28 days.

During the experimental period body weight, food intake was measured daily. At the end of experimental period, blood samples were collected by retro-orbital plexus puncture under light ether anesthesia after 12 hr fasting for biochemical estimation and OGTT was performed.

### 2.4 Oral glucose tolerance test:

The oral glucose tolerance test (OGTT) was performed measuring plasma glucose at the end of the study period. The rats were fasted for 12 hrs. A dose of 2 gm/kg (body weight) glucose solution was given by gastric gavages. Blood samples were obtained from the retro orbital plexus at pre- and 30, 60, and 120 min post-glucose load. Plasma glucose levels were measured by the glucose oxidase reaction method.

### 2.5 Measurement biochemical parameters:

Plasma insulin concentrations were measured by Immuchem Radioimmunoassay method using RIA kit (BI-INSULIN IRMA, Cisbio, France). Total cholesterol, triglyceride and glucose levels were measured spectrophotometrically using commercially available kits according manufacturer instructions (ERBA diagnostic Mannheim GmbH Ltd.). Insulin resistance index was calculated by Homeostasis Model Assessment (HOMA) using the following formula: \[ \text{insulin (µU/ml)} \times \text{glucose (mg/dl)} / 405. \]

### 2.6 Statistical analyses:

All data are presented as Mean ± S.E. The statistical analyses were performed using One-Way ANOVA followed by Dunnett’s multiple Comparison post test. Statistical significance was assumed if p<0.05.

### 3. RESULTS:

#### 3.1 Effect of ASME on Body weight and food intake:

Dexamethasone treatment for 28 days in rats lead to significant decrease in body weight (P<0.0001) when compared to control (Table-1). Similarly, cumulative food intake was significantly less (P<0.0001) in Dexamethasone treated rats when compared to control (Table-1). ASME co-administration show significant improve on Dexamethasone induced reduction in body weight and food intake. (Table-1).

#### 3.2 Effect of ASME on Plasma Glucose, Insulin, Triglyceride and Total cholesterol:

Dexamethasone treatment in rats lead to significant increase in plasma glucose (P<0.0001), plasma insulin (P<0.0001) and triglyceride level (P<0.0001) but had no effect on total cholesterol level when compared to control (Table-1). ASME co-administration significantly prevented the dexamethasone induced increase in plasma glucose (P<0.0001), plasma insulin (P<0.0001), plasma triglyceride (P<0.001), as well total cholesterol level (P<0.0001) when compared to Dexamethasone treated rats (Table-1). The most pronounced antihyperglycemic effect was obtained with dose of 400 mg/kg.

#### 3.3 Effect of ASME on Insulin resistance index (HOMA-IR):

Dexamethasone treatment in rats lead to development of insulin resistance as indicated by significant increase in HOMA-IR index (P<0.0001) when compared to control (Table-1). ASME co-administration in Dexamethasone treated rats significantly reduced the development of insulin resistance as indicated by significant reduction in HOMA-IR index (P<0.0001) when compared to Dexamethasone treated rats (Table-1).

### Figure-1: Effect of ASME (200 mg/kg and 400 mg/kg) on total AUC of plasma glucose after oral glucose challenge in dexamethasone treated rats.

### Figure-2: Effect of ASME (200 mg/kg and 400 mg/kg) on Lipid Peroxidation (plasma MDA level) in dexamethasone treated rats.

Data are expressed as Mean ± S.E. n=7, *p<0.01, when compare to control, **p<0.001, when compare to dexamethasone control.
ministered rats significantly decreased insulin resistance index (P<0.001) (Table-1) when compared to Dexamethasone treated rats.

3.4 Effect of ASME on Oral Glucose Tolerance Test (OGTT):
Dexamethasone treatment in rats lead to glucose intolerance as indicated by significant increase in total AUC of plasma glucose (P<0.01) during 120 min duration of a glucose tolerance test when compared to control (Figure-1). ASME co-administration in Dexamethasone administered rats significantly improved the glucose tolerance (P<0.01) (Figure-1) when compared to Dexamethasone treated rats.

3.5 Effect of ASME on Lipid Peroxidation (plasma MDA level):
Dexamethasone treatment in rats lead to significant increase in plasma MDA level (P<0.001) when compared to control (Table-1). ASME co-administration in Dexamethasone treated rats significantly decreased plasma MDA level (P<0.001) when compared to Dexamethasone treated rats (Table-1).

4. DISCUSSION:
Insulin resistance in humans has been shown to be present in conditions like NIDDM, obesity and dyslipidemia. Thus interventions to decrease insulin resistance may postpone the development of NIDDM and its complications. Treatment with natural herbs is likely to be fraught with lesser side effects compared to the presently used synthetic oral anti diabetic agents. Several studies have brought attention on the treatment with antioxidants might be an effective strategy for reducing diabetic complications due to disproportionate generation of free radicals. In today’s era, several clinical trials prompted new studies focusing on the mechanisms of oxidative stress in diabetes in order to develop causal antioxidant therapy.24

Alangium salvifolium leaves were found to contain flavonoids, terpenoids, alkaloids and steroids which are known to be bioactive antidiabetic principles. Hence, the present study was conducted in order to scientifically validate the therapeutic preparation of this plant in the control of diabetes.

Dexamethasone increases triglyceride levels, causing an imbalance in lipid metabolism leading to hyperlipidemia25 and an increase in glucose levels leading to hyperglycemia.26 Pharmacological doses of glucocorticoids induce ob gene expression in rat adipocyte tissue within 24 h. This is followed by complex metabolic changes resulting in decrease in food consumption; reduction in body weight, profound obesity often accompanied by diabetes and development of insulin resistance with enhanced blood glucose and triglyceride levels. In the present study, low dose dexamethasone for 28 days in rats resulted in increased triglyceride and glucose levels similar to a previous study27. A higher dose of ASME prevented the rise in triglyceride and glucose caused by dexamethasone. Further, ASME also prevented the progressive decrease in body weight caused by dexamethasone.

The ASME might have improved insulin resistance through enhanced insulin sensitivity in peripheral tissues, as was evident from the decreased glucose and insulin and increased liver and skeletal muscle glycogen stores. The drugs ameliorating hyperinsulinemia are likely to have greater therapeutic potential as they may also exert beneficial effects on the clinical course of NIDDM, hypertension and coronary artery disease conditions.

Oxidative stress has been attributed to play a significant role in various diseases including insulin resistance and cardiovascular diseases. Consistence with this notion, dexamethasone administration is associated with systemic oxidative stress. In the present study supplementation of ASME significantly reduced the oxidative stress. These observations suggest that beneficial effects of ASME may be partially due to antioxidant nature of Alangium salvifolium. In conclusion, oral administration of ASME at a dose of 400 mg/kg lowers serum glucose, insulin, triglyceride and total cholesterol concentrations in dexamethasone-administered rats. If these results are extrapolated to humans then ASME might prove useful in the treatment and/or prevention of insulin resistance in nondiabetic states such as obesity and impaired glucose tolerance. However, this notion needs further detail study.

REFERENCES:


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