

# Evaluation of hypoglycemic and antihyperglycemic effect of aqueous-methanolic leaves extract of two medicinal plants of Meghalaya in mice

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## Abstract

The objective of the present study is to evaluate the hypoglycemic and antihyperglycemic effect of aqueous-methanolic extract of *Olax acuminata* and *Bauhinia acuminata* leaves in normoglycemic and alloxan-induced diabetic mice. For hypoglycemic study, normoglycemic mice were administered with varying doses of extracts and the optimal dose was selected for glucose tolerance test. For antihyperglycemic study, the optimal dose of extract was administered to diabetic mice and glucose tolerance test was also performed. In addition, elements in the leaves of plants were also analysed to relate the presence of elements to their antidiabetic property. At the dose of 250 mg/kg b.w., *O. acuminata* leaves extract showed significant reduction in the blood glucose level in normoglycemic and diabetic mice while *B. acuminata* leaves extract showed significant reduction at 500 mg/kg b.w. Glucose tolerance was also improved in both normoglycemic and diabetic mice on administration of the extracts. The results were compared with those of insulin and metformin which were used as standard drugs. Elements found in the leaves of *O. acuminata* were Cu, Cr, Mn, Zn, Fe, V, Mg, K, C, Sr and P whereas *B. acuminata* leaves contain all the above elements except for V and Pb. These mention elements are widely known for regulating blood glucose level. The aqueous-methanolic leaves extract of both the plants demonstrates hypoglycemic and antihyperglycemic effect, thus, could be promising plants in the treatment of diabetes.

**Keywords:** Antihyperglycemic activity, *B. acuminata*, element analysis, hypoglycemic activity, *O. acuminata*

## 1. Introduction

Diabetes is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism [1]. It is one of the most costly, burdensome chronic diseases which require lifelong treatment [2]. Several oral hypoglycemic agents are the primary forms of treatment for diabetes but they have prominent side effects and fail to significantly alter the course of complications related to diabetes [3,4]. This is the main reason for increasing number of people seeking alternative therapies to treat diabetes and one such way is to use medicinal plants as they are considered to be less toxic and free from side effects than synthetic drugs [5,6]. Antidiabetic compounds from natural origin can counter not only the high cost but even poor availability of synthetic drugs especially in developing countries [7]. Thus, plant-based drugs are emerging as primary components of holistic approaches to diabetes management [8]. About 800 plant species have been reported to possess antidiabetic properties [9]. Even the popular hypoglycemic drug glucophage (metformin) is derived from *Galega officinalis* [4]. There are several mechanisms by which antidiabetic plant displayed their medicinal properties, one such study have demonstrated that marked alterations in trace elemental concentrations in the human body are associated with the occurrence of diabetes [10,11]. Hence, regulation of trace elemental concentration is also one of the proposed potential strategies for prevention and treatment of diabetes [12]. The quantitative estimation of trace elements concentration is therefore, important for determining the effectiveness of medicinal plants in treating various diseases and also to understand the pharmacological action [13].

Meghalaya is one of the species rich and mega biodiversity centers in the North Eastern region of India [14], endowed with different types of medicinal plants used for treating number of diseases including diabetes. Several medicinal plants of this region have been found to possess antidiabetic property [15,16]. *Olax acuminata* Benth. (Family: Olacaceae) and *Bauhinia acuminata* L. (Family: Fabaceae) are the medicinal plants available in this region. The leaves of *O. acuminata* are used as cathartic [17] and *B. acuminata* roots decoction boiled with oil is used to treat burns [18]. This present study aimed to elucidate any hypoglycemic and antihyperglycemic effects of the aqueous-methanolic extract of the leaves of these plants using normoglycemic and diabetic mice.

## 2. Materials and Methods

### 2.1 Collection of plant materials

Plant materials were collected from Pomshutia, East Khasi Hills, Shillong, Meghalaya, India. *O. acuminata* (OA) was authenticated at Botanical Survey of India, Eastern Regional Centre, Shillong-793003, Meghalaya (Voucher No. 4975) and *B. acuminata* (BA) was authenticated by a herbarium curator, Dr. P. Gurung, Department of Botany, North-Eastern Hill University (NEHU), Shillong, Meghalaya (Voucher No. NEHU-11859).

### 2.2 Preparation of extract

The leaves were thoroughly washed, dried at 40°C and then powdered. The powder was extracted with 10 volume of aqueous-methanol solution (1:4) using the method of Harborne [19]. The yield percentage of *O. acuminata* extract (OAE) and *B. acuminata* extract (BAE) was found to be 9.50% and 2.80% respectively.

### 2.3 Experimental animals

Female Swiss albino mice (25-30g) were housed in a room kept under control conditions with temperature maintained at 22°C on a 12h dark cycle and fed with standard mice feed, obtained from Pranav Agro Industries Limited (Pune, India). Mice were fasted overnight for each experiment and were given water *ad libitum*. For each study, experiments on six groups of mice were performed. The clearance certificate for research project was approved by the Institutional Ethics Committee (IEC) guidelines of NEHU, Shillong, Meghalaya, India.

### 2.4 Toxicity study

Healthy mice were observed after administration of a single dose of plant extracts: OAE (50-500 mg/kg b.w.)/BAE (250-6000 mg/kg b.w.) via intraperitoneal route (i.p.) for any adverse signs and symptoms at hourly intervals for the next 24h. LD<sub>50</sub> was calculated using the arithmetic method of Karber [20] as modified by Aliu and Nwude [21]. The LD<sub>50</sub> for a particular substance is the amount that can be expected to cause death in half (i.e. 50%) of a group of some particular animal species, usually rats or mice, when administered by a particular route. The formula for LD<sub>50</sub> calculation is given below:

$$LD_{50} = \frac{n}{c - \{(a \times b)\}} \quad \text{eq (1)}$$

$n$  = total number of animal in a group.

$a$  = the difference between two successive doses of administered extract/substance.

$b$  = the average number of dead animals in two successive doses.

$c$  = maximum dose

### 2.5 Hypoglycemic test

Normoglycemic mice were administered via i.p. route with OAE (50-250 mg/kg b.w.)/BAE (250-750 mg/kg b.w.) and their glucose level were measured at 2, 4, 6 and 24h using glucometer. Mice were fed after measuring the blood glucose at 6h [15]. The control mice were given only 2% ethanol.

### 2.6 Induction of diabetes

Hyperglycemia was induced by intravenous administration of alloxan (80 mg/kg b.w.) prepared in acetate buffer (0.15M, pH 4.5) to overnight fasted mice. Mice showing blood glucose levels above 200 mg/dl (>11.1mMol/L), were considered diabetic and were used in this study.

### 2.7 Antihyperglycemic test

Diabetic mice were administered via i.p. route with OAE (250 mg/kg b.w.)/BAE (500 mg/kg b.w.) and blood glucose level was measured at 2, 4, 6 and 24h using a glucometer. Similarly, the test was also performed on diabetic mice using standard drugs i.e., metformin (MET, 250mg/kg b.w.)/insulin (INS, 0.1IU/kg b.w.). Mice were fed after measuring the blood glucose at 6h. The diabetic control mice were given only 2% ethanol.

### 2.8 Glucose tolerance test

Plant extracts/standard drugs were administered via i.p. route to normoglycemic and diabetic mice one and a half hour prior to the oral glucose load of 2 g/kg b.w. [15]. Blood glucose was measured at 30, 60 and 120min after the glucose load [15]. A control group received only the glucose load.

### 2.9 Elemental Analysis

Elements in the leaves of OA was analysed using Graphite furnace-Atomic Absorption Spectrometer (AAS Perkin Elmer 3110) at Sophisticated Analytical Instrumental Facility (SAIF), NEHU, Meghalaya, India and Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (Arcos from M/S. Spectro, Germany), at SAIF, Indian Institute of Technology (IIT), Mumbai, India while the concentration of elements in BA leaves was determine only by ICP-AES at SAIF, IIT, Mumbai.

### 2.10 Statistical analysis

Data were expressed as means ± standard error of the mean (SEM). The statistical analysis was carried out using

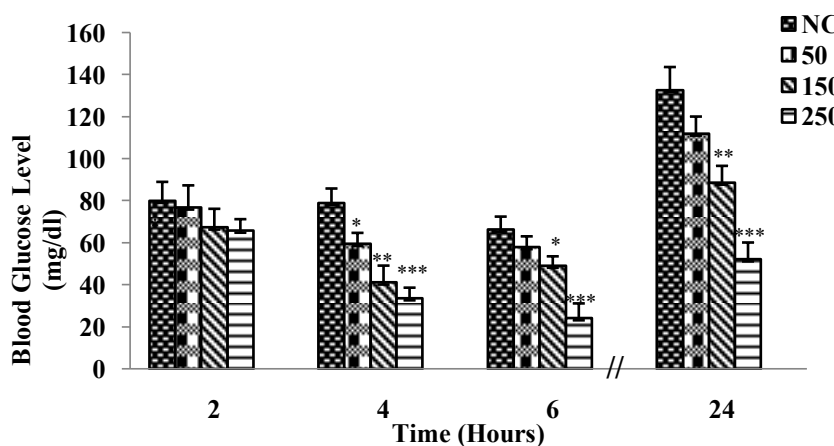
student's t-tests to determine the level of significance. The values  $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$  were considered as statistically significant.

### 3. Results and Discussion

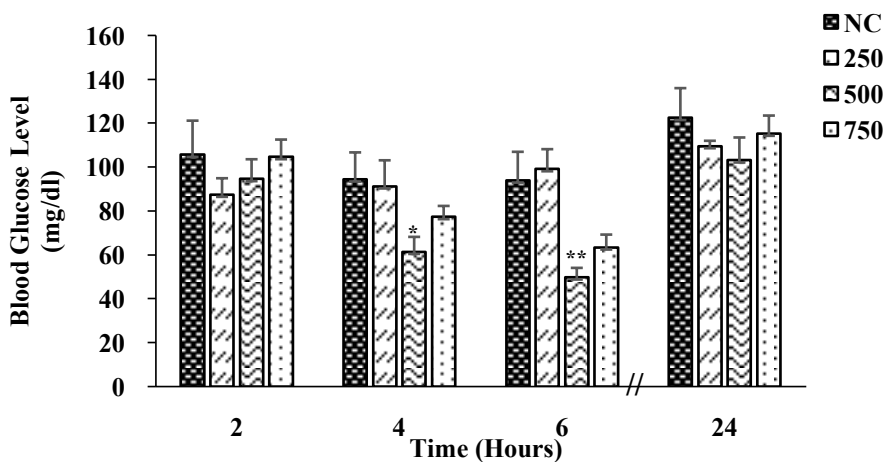
Toxicity studies revealed that the administration of OAE at the dose of 500 mg/kg b.w. caused death of animals within 24h giving LD<sub>50</sub> value of 375 mg/kg b.w. Administration of BAE did not produce significant changes in the behaviour of animals and no death was observed up to the dose of 6000 mg/kg b.w. which may be considered safe as suggested by Lorke [22]. Therefore, in terms of toxicity BAE seems to be safer than OAE. The property of extracting solvents significantly affects the toxicity level of plant extract as typical properties of good solvents include low toxicity [23]. Hence, changing the extracting solvent for *O. acuminata* leaves may offer a safer extract, for which further investigation would be required.

Hypoglycemic test showed that the i.p. treatment of OAE decreased the blood glucose level in a dose-dependent manner (figure 1). Among the doses, 250 mg/kg b.w. was shown to be more effective at 24h by 60.68% ( $p < 0.001$ ) when compared to the control group. Although, the blood glucose level at 24h was at a higher range than observed at 2, 4 and 6h in both the normoglycemic control and treated groups. This probably may be due to the fact that mice were fed after 6h treatment in order to prevent death due to hypoglycemia that would likely occur in overnight fasted animals. Similar response has also been shown in previous studies [15,16].

Figure 2 show that BAE treatment decreased the blood glucose in a dose-independent manner. At 500 mg/kg b.w., BAE showed highest reduction in blood glucose level at 6h by 46.98% ( $p < 0.01$ ) when compared to the control group. The obtained result indicates that the aqueous-methanolic leaves extract of both the plants possessed hypoglycemic activity. Several plants with hypoglycemic effect in normoglycemic mice have also shown to possess antihyperglycemic activity in diabetic mice [15,16]. Therefore, the dose of 250 mg/kg b.w. of OAE and 500 mg/kg b.w. of BAE were further selected for determining their antihyperglycemic effect in diabetic mice.

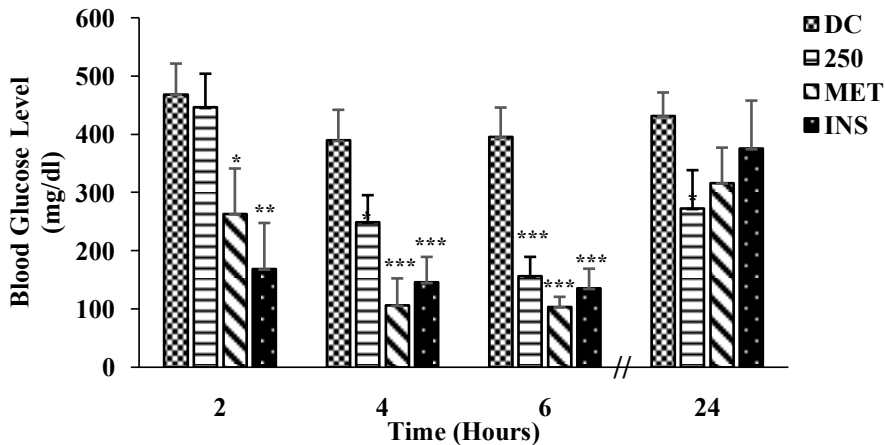


**Figure 1:** Effect of varying doses (represented as mg/kg b.w.) of OAE on blood glucose level of normoglycemic mice where (NC) is normoglycemic control. Values are expressed in mean± SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

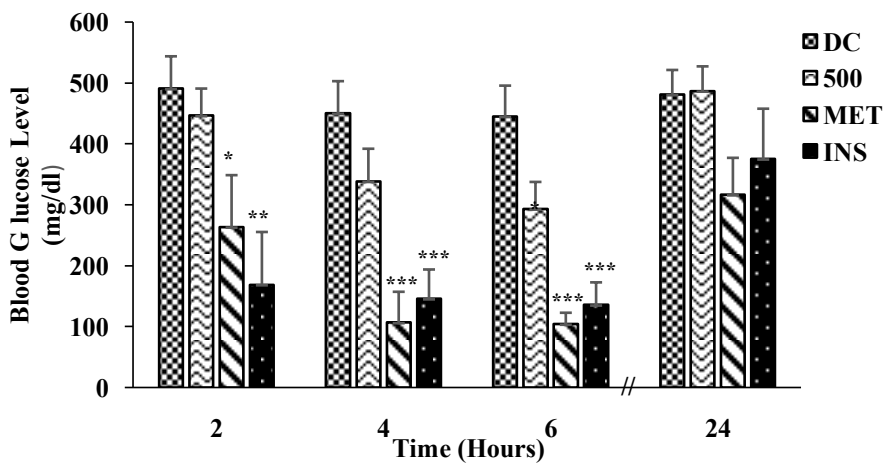


**Figure 2:** Effect of varying doses (represented as mg/kg b.w.) of BAE on blood glucose level of normoglycemic mice where (NC) is normoglycemic control. Values are expressed in mean ± SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Alloxan is a drug use to induce experimental diabetes due to the selective destruction of the insulin-producing pancreatic beta-cells [24]. The elevated level of blood glucose observed in alloxan-treated mice confirmed the diabetic state which may be attributed to the selective cytotoxicity effect of alloxan on the beta cells. Treatment with OAE lowered the rise in blood glucose level in diabetic mice and showed highest antihyperglycemic effect at 6h by 64.74% ( $p<0.001$ ) when compared to the diabetic control (figure 3). Extract of BA showed pronounced antihyperglycemic effect at 6h (34.13%;  $p<0.05$ ) when compared to the diabetic control (figure 4). MET and INS treated diabetic mice showed highest reduction in blood glucose at 4h (76.33%;  $p<0.001$ ) and at 2h (65.58%;  $p<0.001$ ) respectively when compared to the diabetic control (figure 3 and 4). The data obtained clearly indicate that the aqueous-methanolic extract of both the plants have antihyperglycemic effect in diabetic mice.



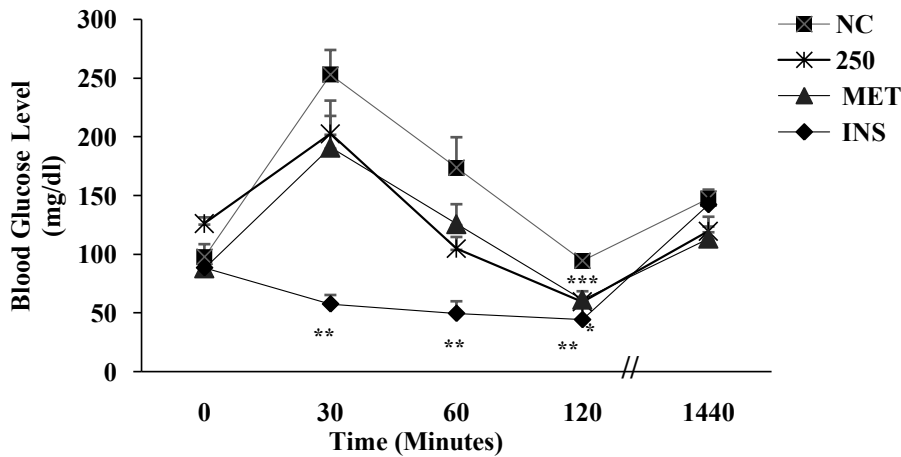
**Figure 3:** Effect of OAE (250 mg/kg b.w.)/standard drugs on blood glucose level of diabetic mice where DC is diabetic control. Values are expressed in mean  $\pm$  SEM. \* $p<0.05$ , \*\* $<0.01$ , \*\*\* $<0.001$ .



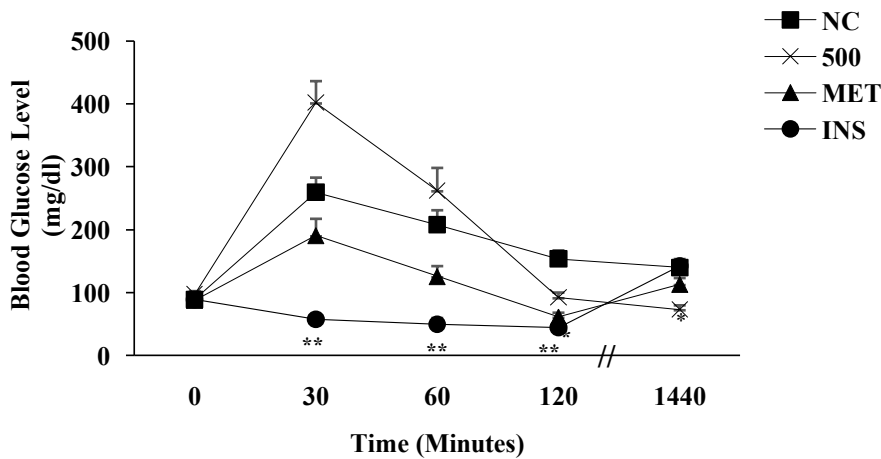
**Figure 4:** Effect of BAE (500 mg/kg b.w.)/standard drugs on blood glucose level of diabetic mice where DC is diabetic control. Values are expressed in mean  $\pm$  SEM. \* $p<0.05$ , \*\* $<0.01$ , \*\*\* $<0.001$ .

Glucose tolerance test measures the body ability to use glucose, the body’s main source of energy [25]. This test can be used to diagnose pre-diabetes and diabetes. Glucose tolerance test of OAE and BAE in normoglycemic mice is shown in figure 5 and 6. Half an hour after glucose load, marked increase in the blood glucose level of the control and treated mice was observed. Administration of OAE reduced the blood glucose level significantly at 120min by 52.90% ( $p<0.001$ ) whereas BAE lowered the glucose peak by 25.25% ( $p<0.05$ ) at 1440min when compared to the baseline glucose level (0min). Mice treated with MET and INS significantly lowered the glucose peak at 120min by 30.25% ( $p<0.05$ ) and 49.72% ( $p<0.01$ ) respectively. Figure 7 and 8 shows the glucose tolerance test of OAE and BAE respectively in diabetic mice. Blood glucose lowering effect was seen to continue in OAE treated mice even at 1440min (by 49.68%,  $p<0.001$ ) of extract administration. On the other hand, BAE decreased the blood glucose level by 24.58% (not significant) at 120min when compared to 0 min. The blood glucose level of mice treated with MET and INS was reduced by 55.61% ( $p<0.001$ ) and by 70.30% ( $p<0.001$ ) respectively at 120min when compared to 0 min. Therefore, glucose lowering effects were found after administration of OAE and BAE in both normoglycemic and diabetic mice. However, glucose tolerance test was more improved in OAE treated mice than in those treated with BAE. Both the plant extracts showed different pattern of blood

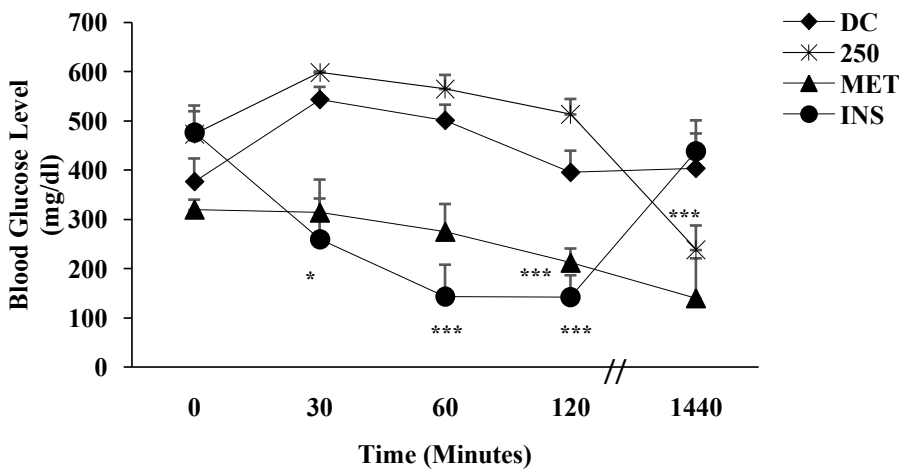
glucose reduction which was not comparable to the standards used. This may suggest that the blood glucose lowering ability of plant extracts follow a different mechanism from those of the standards [26]. Hence, the exact mechanism(s) by which the given extracts induced these effects remains to be investigated.



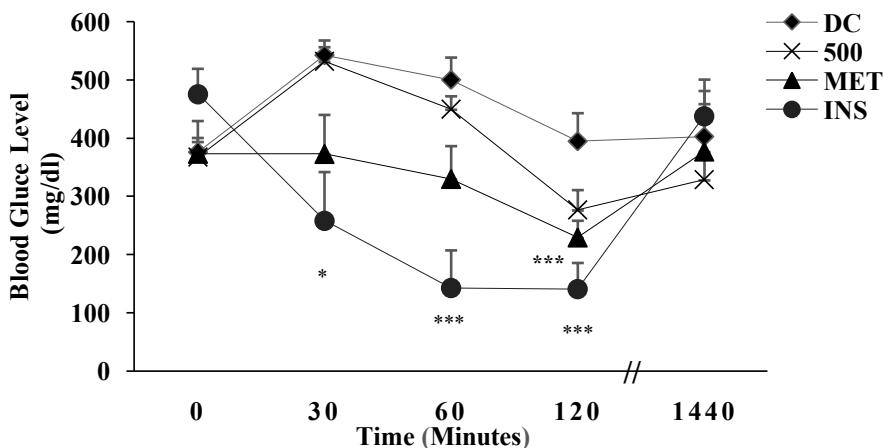
**Figure 5:** Glucose tolerance test in normoglycemic mice administered with OAE (250 mg/kg b.w.)/standard drugs where NC is normoglycemic control. Values are expressed in mean ± SEM. \*p<0.05, \*\*<0.01, \*\*\*<0.001.



**Figure 6:** Glucose tolerance test in normoglycemic mice administered with BAE (500 mg/kg b.w.)/standard drugs where NC is normoglycemic control. Values are expressed in mean ± SEM. \*p<0.05, \*\*<0.01, \*\*\*<0.001.



**Figure 7:** Glucose tolerance test in diabetic mice administered with OAE (250 mg/kg b.w.)/standard drugs where DC is diabetic control. Values are expressed in mean ± SEM. \*p<0.05, \*\*<0.01, \*\*\*<0.001.



**Figure 8:** Glucose tolerance test in diabetic mice administered with BAE (500 mg/kg b.w.)/standard drugs where DC is diabetic control. Values are expressed in mean ± SEM. \*p<0.05, \*\*<0.01, \*\*\*<0.001.

In the present study, elements in the leaves of *O. acuminata* and *B. acuminata* were also determined (table 1-3). Our results show that the leaves of OA analysed by AAS contain elements with level in the decreasing order Mg>Fe>V>Cu>Zn>Mo>Pb>Cr>Mn and Ca>K>P>Sr>Ti were found to be present as per ICP-AES analysis. BA leaves contain Ca>K>Mg>P>Fe>Sr>Zn>Mn>Cu>Ti>Cr as detected by ICP-AES. Reports have shown that plants used for the treatment of diabetes mellitus are endowed with trace elements that possess antidiabetic properties. *Cassia auriculata*, *Gymnema sylvestris*, *Adhathoda vasica*, *Withania somnifera*, *Tinospora cordifolia*, *Azadirachta indica* [27] to name few are antidiabetic plants rich in the aforementioned elements. Mg has been reported to play a role in glucose transport mechanism and is present in various enzymes involved in glucose oxidation pathways [28, 29]. Ca and K are required for INS secretion and plays important role in lowering blood glucose level [30]. V has been reported to mimic the metabolic effects of INS in rat adipocytes [31]. Fe is known for facilitating the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes [32]. Zn is a cofactor for insulin and has been found to influence various enzymes of carbohydrate metabolism [33]. Cu and Mn are known to protect insulin secreting pancreatic β-cells which are sensitive to free radical damage [34]. Mo can regulate blood glucose levels by mimicking insulin activity [35,36]. Cr stimulates insulin signaling pathway and up regulates glucose transporter (GLUT4) translocation in muscle cells [37]. P mainly exists as phosphate which on depletion impairs INS secretion and is associated with resistance to the peripheral action of insulin and with glucose intolerance [38]. Not much is known about biological role of Ti and Sr. Therefore, the presence of these elements might have played a direct or indirect role in reducing the blood glucose level.

As mentioned above, OA leaves sample was found to be rich in Mg (0.43 ppm) and Ca (1.158%) respectively by AAS and ICP-AES method. The Mg and Ca level in OA leaves is comparable with antidiabetic plants, *Bauhinia tomentosa* [39,40] and *Pueraria tuberosa* [41,42] respectively. BA leaves sample revealed that Ca level (1.769%) was higher compared to other elements as detected by ICP-AES which was comparable to the level found in antidiabetic plant, *Costus pictus* [43]. All the trace elements found in both the plants were within the permissible limits [44].

**Table 1: Trace elements in the leaves of *O. acuminata* using AAS**

Elements (ppm)	Mg	Fe	V	Cu	Zn	Mo	Pb	Cr	Mn
	0.43	0.25	0.25	0.20	0.12	0.10	0.09	0.08	0.070
	±0.006	±0.001	±0.002	±0.004	±0.001	±0.004	±0.00	±0.002	±0.001

Data are expressed in ppm.

**Table 2: Trace elements in the leaves of *O. acuminata* analysed by ICP-AES**

Elements (%)	Ca	K	P	Sr	Ti	Se	As
	1.158	0.965	0.175	0.0018	0.00027	ND	ND

Data are expressed in percentage. ND (Not detectable).

**Table 3: Trace elements in the leaves of *B. acuminata* analysed by ICP-AES**

Elements (%)	Ca	K	Mg	P	Fe	Sr	Zn	Mn
	1.769	0.821	0.342	0.16	0.0094	0.0036	0.0028	0.001
Elements (%)	Cu	Ti	Cr	As	Mo	Pb	Se	V
	0.00078	0.00011	0.000079	ND	ND	ND	ND	ND

Data are expressed in percentage. ND (Not detectable).

#### 4. Conclusion

The present study revealed that the aqueous-methanolic leaves extract of *O. acuminata* and *B. acuminata* possess both hypoglycemic as well as antihyperglycemic effect. Both the extracts enhanced glucose utilization and improve tolerance in glucose loaded normoglycemic and diabetic mice.

#### Acknowledgement

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