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# PHCOG MAG.: Research Article
# Hypoglycemic and antihyperglycemic effect of alcoholic extract of *Euphorbia leucophylla* and its fractions in normal and in alloxan induced diabetic rats

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**ABSTRACT** - The hypoglycemic and antihyperglycemic effect of alcoholic extract of Euphorbia leucophylla was investigated in normal and alloxan induced diabetic rats. A single oral administration of alcoholic extract at doses 100, 250 and 500 mg $kg^{-1}$ produced a significant blood glucose reduction in a dose dependent manner and elevated the serum insulin levels in normal and diabetic rats. A bioguided extraction and fractionation of alcoholic extract of the roots of E. leucophylla afforded a new flavone xylopyranoside, 4', 5-Dihyroxy-6, 7-dimethoxy flavone-3-O-β-D-xylopyranoside (ELR-05), identified as active principle together with the known compounds taraxerol, taraxerone, lupeol, 3-O-(z)-p-coumaroyl taraxerol. The isolated compound, 4', 5-Dihyroxy-6, 7-dimethoxy flavone-3-O-β-D-xylopyranoside obtained by ethylacetate fraction of alcoholic extract at doses 25, 50 and 75 mg/kg significantly reduced the blood glucose levels and increased the serum insulin levels in normal and diabetic rats. These data confirm the hypoglycemic and antihyperglycemic effect of alcoholic extract and its active isolated compound (ELR-05) in normal and diabetic rats.

**KEYWORDS**- Diabetes mellitus; *Euphorbia leucophylla*; Alloxan; Flavone xylopyranoside

## INTRODUCTION

Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality(1).Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries (2).These may be delayed, lessened or prevented by maintaining blood glucose values close to normal. The increasing number of ageing population, consumption of calories rich diet, obesity and sedentary life style have led to a tremendous increase in the number of diabetics world-wide. According to WHO projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025 making it the country with the highest number of diabetics in the world (3,4). Therefore, it is necessary to look for new solutions to manage this health problem. In modern medicine, the beneficial effects on glycemic levels are well documented; the preventing activity of these drugs against progressive nature of diabetes and its micro and macrovascular complications was modest and not always effective. Insulin therapy affords effective glycemic control, yet its short comings such as ineffective-ness on oral administration, short shelf life, requirement of constant refrigeration, and in the event of excess dosage — fatal hypoglycemia —limits its usage. Treatment with sulfonylureas and biguanides is also associated with side effects (1). It is apparent that due to the side effects of the currently used drugs, there is a need for a safe agent.

with minimal adverse effects, which can be taken for long durations. Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine to natural products that may be better treatments than currently used drugs (5) In the indigenous system of medicine (Ayurveda), a mention was made on good number of plants for the cure of diabetes or 'Madhumeha' and some of them have been experimentally evaluated and

the active principles were isolated (6,7,8). However, search for newer antidiabetic drugs continues.

The Euphorbiaceae family is one of the largest families in the plant kingdom. It comprises 263 genera and about 7300 species of almost cosmopolitan distribution. Euphorbia, the largest genus of Euphorbiaceae, with about 1600 species is characterized by the presence of milky latex. This genus has been the subject of numerous chemical studies (9, 10, 11). Plants belonging to *Euphorbia* species have been the subjects of many investigations for their biologically active components. Biological activities including skin irritant, tumor promotion and proinflammatory properties are attributed to the presence of specific classes of macro- and polycyclic diterpenes (12, 13, 14). Some species of Euphorbia have been used as medicinal plants for the treatment of skin diseases, gonorrhea, migraine, intestinal parasites, wart cures (15) and as anti-inflammatory in Indian folk medicinal system. However, no chemical investigation on *Euphorbia leucophylla* has been reported up to now. In the present study, we report the isolation and structure elucidation of a new flavone xyloside namely 4', 5-Dihyroxy-6, 7-dimethoxy flavone-3-o-β-D-xylopyranoside (compound ELR-05) from roots of *E. leucophylla*. In addition, taraxerol, taraxerone, lupeol, 3-(z)-p-coumaroyl taraxerol were isolated.

## MATERIALS AND METHODS

### *Plant material*

The roots of the plant *Euphorbia leucophylla* was purchased from local nurseries and identified by Dr. M. Venkaiah, Associate Professor, Department of Botany, Andhra University. A voucher specimen (MN-108) is deposited in herbarium of our Department, Andhra University, Visakhapatnam.

### *Animals*

Laboratory bred Sprague Dawley rats of either sex weighing 200-225 g were employed for the study. All animals were procured from National Institute of Nutrition, Hyderabad. The rats were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 50 ± 15% and normal photo period [12 h dark / 12 h light] were used for the experiment. Commercial pellet diet [Ratan Brothers, India] and water were provided *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics committee and by the Regulatory body of the government [Regd no.516/01/A/CPCSEA].

### *Drugs*

Alloxan monohydrate was purchased from sigma chemicals (St Louis, USA). All other chemicals used for this study were of analytical grade

### *Extraction and isolation procedure*

Freshly collected roots of *E. leucophylla* were cut into small pieces and shade dried. The dried roots were powdered in Wiley mill. The powdered root (1500 g) was extracted with ethyl alcohol (95% v/v) by process of continuous extraction (Soxhlation). The crude extract was evaporated to dryness in a rotary film evaporator. The yield of dried extract was 48g. The alcoholic extract was fractioned with hexane (10x250 ml), ether (4x150ml), chloroform (7x150ml), ethylacetate (4x150ml) and methanol (15x200ml). All fractions were concentrated and the percentage yields were: hexane 3%, ether 10%, chloroform 20%, methanol 1% and ethylacetate 1% (w/w) in terms of dry plant material. While concentrating the ethylacetate layer, a yellow compound was precipitated and it was filtered under vacuum and the filtrate was concentrated, purified and noted as a compound ELR-05 (yield 18.33%). Among all the fractions, the hexane fraction showed four distinct spots in thin layer chromatography over silica gel in different solvent systems and the hexane layer was chromatographed over silica gel (finer than 200#, ACME) column and eluted taking 1000 ml fractions, starting with hexane/ EtoAc, 90:10 (frs. 20-24) to afford compound ELR-01 (yield 0.011%), Hexane/EtoAc, 90:10(frs. 25-30) to afford compound ELR-02 (yield 0.016%), hexane/EtoAc , 85:15 (frs 31-34) to afford compound ELR-03 (yield 0.012%), hexane/EtoAc , 80:20 (frs. 37-40) to afford compound ELR-04 (yield 0.026%). Compound ELR-01, ELR-02, ELR-03 and ELR-04 were identified as taraxerol, taraxerone, lupeol and 3-(z)-p-coumaroyl taraxerol respectively and by comparing the data with known literature values of NMR and IR. Compound ELR-05 was identified as 4, 5-Dihyroxy-6,7-dimethoxy flavone-3-O-β-D-xylopyranoside.

### *Toxicity evaluation in mice*

The alcohol extract was tested for its acute and short-term toxicity (if any) in mice. To determine acute toxicity, a single oral administration of the alcoholic extract at doses of 0.25, 0.5, 0.75 and 1.0 gm/kg were administrated to different groups of mice (5 mice were used for each group, control mice received Tween 80). Mortality and general behavior of the animals were observed periodically for 48 h. The animals were observed continuously for the initial 4 h

and intermittently for the next 6 h and then again at 24 h and 48 h following administration of the plant extract. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion. To study short-term toxicity, 3 groups of mice each containing 6 male mice (20-25 g, body weight) were used. Group 1 was kept as control and Group II and III received 200 and 400 mg/kg alcoholic extract respectively in 5% Tween 80. The extract was administrated daily for 14 days (p.o.). Control group received 5% Tween 80 in an identical manner. The behavior of the animals was observed daily for 1h in the forenoon (10 to 11 A.M.) for 14 days. Initial and final body weights, water and food intake, state of stool and body temperatures were observed.

Induction of diabetes

Animals were allowed to fast 18 h and were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 140 mg/kg body weight intraperitoneally. After 2 weeks, diabetic rats (250-350 mg/dl) were used for the experiment.

***Experimental design***

In the experiment a total number of 80 rats (40 normal and 40 diabetic surviving rats) were used. The rats were divided into 16 groups (8 normal and 8 diabetic), each group consisting of 5 animals. Group 1 normal rats treated with vehicle (1% Sodium CMC) and served as normal control, and Group 2, 3 and 4 normal rats were treated with alcoholic extract of *E. leucophylla* at a doses of 100, 250, and 500 mg/kg respectively, Group- 5, 6 and 7 normal rats treated with compound ELR-05 at doses 25, 50 and 75 mg/kg dose respectively. Group 8 normal rats treated with tolbutamide (40 mg/kg) served as standard reference. Group 9 diabetic rats treated with vehicle and served as diabetic control. Group- 10, 11and 12 diabetic rats were treated with alcoholic extract of *E. leucophylla* at doses of 100, 250, and 500 mg/kg respectively; Group- 13, 14 and 15 diabetic rats were treated with compound 5 at doses 25, 50 and 75mg/kg dose respectively. Group- 16 diabetic rats treated with 40 mg/kg dose of Tolbutamide. All the doses were administered orally.

***Oral glucose tolerance test***

After overnight fasting, 0 min blood sample (0.2 ml) was taken from the rats in normal control, diabetic control, normal + plant extract treated group, normal + compound ELR-05 treated, diabetic + plant extract treated, diabetic + compound ELR-05 treated groups by orbital sinus puncture(16). Glucose solution (2 g/kg) was administered orally immediately. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration (17).

***Estimation of blood glucose and serum insulin levels***

The rats were fasted for 18 h and blood samples were collected by puncture of retro-orbital plexus immediately with capillary tube under ether anesthesia into glass vials containing a small quantity of a mixture of potassium oxalate and sodium fluoride as an anticoagulant at 0 h (before treatment) and 2, 4, 6, 8, 12, 16, 24 h (after treatment). The plasma blood glucose levels were determined by using GOD--POD method (18). Serum insulin was determined by Radio Immunoassay (RIA) method.

***Statistical analysis***

All values were expressed as Mean ± SEM. The differences were compared using one-way analysis of variance (ANOVA) followed by Dunnet's *t* test. P values <0.05 were considered as significant.

**RESULTS**

Compound ELR-01: colourless needles from $CHCl_3$: MeOH, m.p. 280-282$^0$c, [$\alpha$]+0.78$^0$c ($CHCl_3$, c1.14). The molecular formula was determined to be $C_{30}H_{50}O$ by HR–MS. It gave a positive Liebermann- Burchard test and readily formed a monoacetate, m.p. 292-294$^0$c. The $^1$H as well as $^{13}$C NMR- data is in agreement with those already reported earlier for taraxerol (19,20,21). $^{13}$C NMR spectrum of ELR-01 acetate was found to be identical with spectrum of taraxerol acetate.

**1. Compound ELR-02:** colourless needles from MeOH, m.p.238-240$^0$c, [$\alpha$]+ 11.6$^0$C ($CHCl_3$, c 0.78). The molecular formula was determined to be $C_{30}H_{48}O$ by HR–MS. The $^1$H as well as $^{13}$C NMR- data was found to be identical with spectrum of those already reported earlier for taraxerone (21, 22, 23). Compound ELR-03: colourless needles from $CHCl_3$, m.p.210-212$^0$c, [$\alpha$] +27$^0$C ($CHCl_3$, c 0.985). The molecular formula was determined to be $C_{30}H_{50}O$ by HR–MS. The $^1$H as well as $^{13}$C NMR- data was found to be identical with spectrum of those already reported earlier for lupeol (23,24,25,26).

**Compound ELR-04:** Amorphous powder from $CHCl_3$, m.p. 284$^0$C. The molecular formula was determined to be $C_{39}H_{56}O_3$ by HR–MS. It gave a violet colour changing to purple in Liebermann- Burchard test. It gave monoacetate, m.p. 248$^0$c. The $^1$H as well as $^{13}$C NMR spectrum of ELR-04 and ELR-04 acetate was identical with those already reported earlier for taraxerol. $^{13}$C NMR spectrum of ELR-01 acetate was found to be identical with spectrum of 3-o-(z)-p-coumaroyl taraxerol and its acetate respectively (27).

**Compound ELR-05:** Yellow amorphous powder, m.p.193-194$^0$C. The molecular formula was determined to be $C_{39}H_{56}O_3$ by HR-MS, IR $\nu_{max}$ ($CHCl_3$) $cm^{-1}$; 3350, 1640, 1620, 1595, 1295, 1215, 1085, 1060; MS m/z (rel. int.): 330 ($M^+$, 100) ($C_{22}H_{22}O_{11}$), 312 (30) ($C_{22}H_{22}O_{10}$), 301 (10) ($C_{21}H_{21}O_{10}$), 287 (95) ($C_{20}H_{19}O_{10}$) 269 (22), 258 (6.6), 244 (15.5), 216 (5), 187 (7.2), 165 (12.4), 153 (10.0), 137 (5), 121 (33.3), 106 (7.6), 93 (11.7), 69 (16.5). $^1$H NMR (pyridine-$d_s$): δ 3.43 (1H,*dd,* J=11.7, 8.5 Hz, H-5"b), 3.51 (1H, *dd,* J=7.7, 6.3 Hz, H-2"), 3.59 (1H, *dd,* J=7.7, 7.7 Hz, H-3"), 3.61 (1H, *m,* H-4"), 3.83 (3H, *s,* 7-$OCH_3$), 3.95 (3H, *s,* 6-$OCH_3$), 4.00 (1H, *dd,* J=11.7, 4.6 Hz, H-5"a), 4.95 (1H, *d,* J=6.3 Hz, H-1"), 6.64 (1H, *s,* H-8), 7.20 (2H, *d,* J=8.0 Hz, H-3', 5'), 7.50 (1H, *s,* 4-OH), 8.40 (2H, *d,* J=8.0 Hz, H-2', 6'), 13.13 (1H, *s,* 5-OH). $^{13}$C NMR (pyridine-$d_5$, 100 MHz): δ 158.2 (C-2), 135.5 (C-3), 179.5 (C-4), 153.5 (C-5), 132.2 (C-6), 159.8 (C-7), 91.7 (C-8), 153.0 (C-9), 107.0 (C-10), 122.3 (C-1'), 132.4 (C- 2',6'), 116.5 (C-3', 5'), 162.2 (C-4'), 104.8 (C-1"), 73.7 (C-2"), 75.7 (C-3"), 70.2 (C-4"), 66.6 (C-5"), 60.9 (6-$OCH_3$), 56.8 (7-$OCH_3$). The structures of compounds were given in Fig.1.

***Effect on normal rats***

The effect of different doses of alcoholic extract of *E. leucophylla* on fasting blood sugar levels in normal rats were assessed at different time interval. The maximum percentage blood glucose reduction with 100, 250 and 500 mg/kg doses of *E. leucophylla* at 8 h were 32.69%, 40.08% and 47.49% respectively. Where as the treatment with the compound ELR-05 at a dose of 25, 50, 75 mg/kg produced maximum percentage reduction at 6h and were found to be 38.53%, 41.40% and 46.69% respectively. Tolbutamide 40 mg/kg dose produced 44.44% blood glucose reduction at 6 hr in normal rats and the results are shown in Table 1. In normal control animals, the serum insulin levels were found to be 16.12 ± 0.56 μU/ml at 8 h, where as the treatment of alcoholic extract of *E. leucophylla* and compound ELR-05 at dose of 500 mg and 50 mg /kg produced a significant increase in serum insulin levels and were found to be 40.13 ± 1.32 μU/ml and 41.13 ± 0.98 μU/ml respectively at 8 h when compared to control group. Similarly tolbutamide 40 mg/kg dose produced a significant rise in serum insulin level at 8 h in normal rats

***Effect on alloxan induced diabetic rats***

The antihyperglycemic effect of different doses of alcoholic extract of *E. leucophylla* on fasting blood glucose levels in diabetic rats were assessed at different time intervals. The percentage blood glucose reduction with 100, 250 and 500 mg/kg dose of *E. leucophylla* at 6 h were 33.03%, 41.79% and 48.00% respectively. Where as the treatment with compound ELR-05 at a dose of 25, 50, 75 mg/kg produced maximum percentage reduction at 6h and were found to be 46.46%, 50.19% and 52.24% respectively. Tolbutamide (40 mg/kg) produced 48.65% blood glucose reduction in alloxan induced diabetic rats and the results are shown in table 2. In diabetic control animals, the serum insulin levels at 8 h were found to be 4.95 ± 0.43 μU/ml, the treatment of alcoholic extract of *E. leucophylla* and compound ELR-05 at a dose of 500 mg and 50 mg/kg produced a significant rise in serum insulin level at 8 h and were found to be 20.18 ± 1.35 μU/ml and 19.98 ± 0.93 μU/ml respectively, when compared to control group. Similarly tolbutamide 40 mg/kg dose produced a significant rise in serum insulin level at 8 h in and alloxan induced diabetic rats.

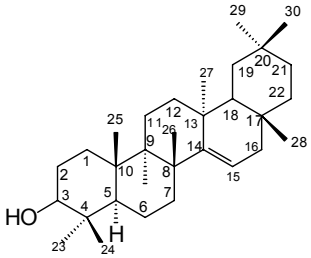
***Toxicity evaluation***

Although it is the normal practice to determine the $LD_{50}$ value, now it is acceptable to limit the study with an acute toxicity test using several doses including reasonably high doses of the drugs. In the present study, acute toxicity was tested up to a high concentration of 1 gm/kg (two times more than the active dose). Even at this dose the herbal extract did not exhibit any sign of toxicity. Since the main purpose of the preliminary acute toxicity study is to get some idea on conspicuous behavioral changes and death, if any, and the alcoholic extract of *E. leucophylla* did not exhibit any toxic symptoms in the limited toxicity evaluation in male mice. Daily feeding for 14 days with the alcohol extract (200 and 400 mg/kg) did not result in any change in general behavior of the animals. Body temperature and state of the stool were also not influenced by the drug treatment. Body weight, weight of liver, kidneys and spleen and food and water intake were not significantly altered by the drug administration

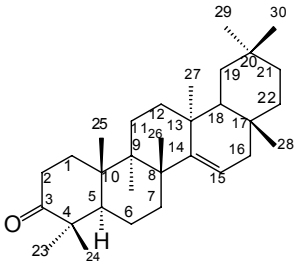
***Effect on oral glucose tolerance test***

Administrations of the alcoholic extract of *E. leucophylla* (500 mg/kg) and compound ELR-05 (50 mg/kg) orally half an hour prior to glucose load showed improved glucose tolerance in normal rats. In control animals the percentage increase in blood glucose at 60 min are found to be 83.15%, where as the treatments with alcoholic extract (500 mg/kg) and compound ELR-05 (50 mg/kg) ameliorates the percentage increase in blood glucose significantly and the results were found to be 38.15% and 41.67% respectively at 60 min in normal rats. Glucose tolerance in alloxan-induced

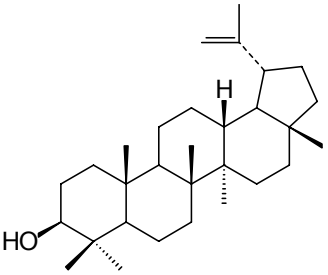
diabetic rats exhibited a similar pattern to that of normal rats and the changes in the levels of blood glucose in diabetic control and experimental groups after oral administration of glucose (2 g/kg) were shown in Table 4.



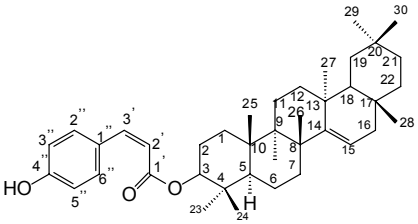
*Taraxerol (ELR-01)*



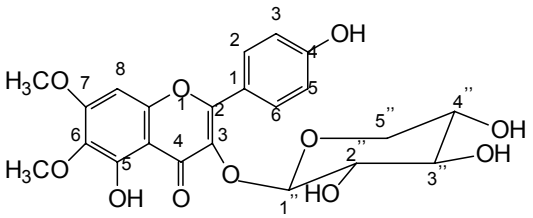
*Taraxerone (ELR-02)*



*Lupeol (ELR-03)*



*3-O-(Z) p-Coumaroyl taraxerol (ELR-04)*



*4′, 5-Dihydroxy-6,7-dimethoxy flavone-3-O-β-D-xylopyranoside (ELR-05)*

***Fig.1. Structures of compounds ELR-01 to ELR-05***

**DISCUSSION**

The present study was conducted to evaluate the hypoglycemic and antihyperglycemic activity of *E. leucophylla* a herbal drug, first identified by us, to get a berth in the group of antidiabetic herbal drugs. In this study, the alcoholic extract of *E. leucophylla* produced a dose dependent percentage blood glucose reduction in normal and diabetic groups. In normal treated groups a significant percentage blood glucose reduction was observed up to 18 h and maximum percentage blood glucose reduction was observed at 8 h, where as in diabetic groups a significant reduction in blood glucose was maintained up to 24 h and maximum at 6 h. The percentage blood glucose reduction produced by the extract in diabetic group is greater than the percentage reduction observed in normal treated groups and *E. leucophylla* (500 mg/kg) dose produced better percentage blood glucose reduction than the tolbutamide in normal and diabetic groups. The treatment with compound ELR-05 (50 mg/kg) produced a maximum percentage blood glucose reduction at 6 h in normal and diabetic rats.

Different mechanisms of action to reduce blood glucose levels with the help of plant extracts already exist. Some plants exhibit properties similar to the well-known sulfonylurea drugs like tolbutamide; they reduce blood glucose in normoglycemic animals (28,29).

Some other plants act like biguanides such as metformin, which is an antihyperglycemic compound; they do not affect blood glucose in normal state (30, 31, 32, 33). We hypothesized that *E. leucophylla* could have a sulfonylurea-like mechanism since it decreased blood glucose in normoglycemic rats such as tolbutamide. It is also known that alloxan selectively destroys insulin-secreting β-cells in the islets of Langerhans and their effects are irreversible (34). In the present study, the dose of alloxan (140 mg/kg, i.p.) was selected in order to partially destroy the pancreatic β-cells. In these conditions, insulin was secreted but not sufficiently to regulate the blood glucose. Sulfonylurea compounds lower blood glucose in normal and type 2 diabetic animals by stimulating insulin release from β-pancreatic cells. In the present study, the treatments with alcoholic extract of *E. leucophylla* (500 mg/kg) and compound ELR-05 (50 mg/kg) significantly elevated the serum insulin levels in normal and alloxan induced diabetic rats. Study on underlying cellular mechanism by which the alcoholic extract of *Euphorbia leucophylla* stimulate the secretion of insulin from β-cells, cannot be defined.

***Table 1 - Effects of oral administration of alcoholic extract of E. leucophylla roots on fasting blood glucose level in normal rats.***

| Group | Dose (mg/kg) | Blood glucose levels (mg/dl) at different hours | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 h | 2 h | 4 h | 6 h | 8 h | 12 h | 18 h | 24 h |
| Control | ----- | 87.90±2.05 | 86.12 ± 0.9 (2.02) | 85.50±1.34 (2.73) | 82.15±0.95 (6.54) | 79.28±1.80 (9.80) | 79.66±1.74 (9.37) | 78.74±2.23 (10.42) | 78.34±2.10 (10.87) |
| *E. leucophylla* | 100 | 88.23±1.65 | 84.13±1.20 (4.64) | 75.13±1.97 (14.84)* | 63.15±1.35 (28.42)* | 59.38±1.50 (32.69)* | 61.32±0.95 (30.49)* | 69.39±1.09 (21.35)* | 78.45±0.97 (11.08) |
| | 250 | 90.30±1.97 | 81.15±1.79 (10.13) | 66.57±1.77 (26.27)* | 57.12±1.43 (36.74)* | 54.1±1.12 (40.08)* | 63.16±1.32 (30.05)* | 74.65±1.41 (17.33)* | 80.17±0.85 (11.21) |
| | 500 | 89.77±0.97 | 74.20±1.38 (17.34) | 61.44±1.52 (31.55)* | 51.41±1.06 (42.73)* | 47.13±1.69 (47.49)* | 58.70±0.29 (34.61)* | 68.35±2.69 (23.86)* | 74.17±3.12 (17.37)* |
| ELR-05 | 25 | 85.51±0.58 | 74.03±0.69 (3.42) | 64.85±0.90 (24.16)* | 55.12±2.13 (35.53)* | 61.31±0.60 (28.30)* | 69.24±1.58 (19.02)* | 75.13±0.66 (12.13) | 84.68±0.42 (0.97) |
| | 50 | 87.96±1.22 | 72.81±1.25 (17.22) | 62.20±1.31 (29.28)* | 51.54±1.48 (41.40)* | 58.90±1.30 (33.03)* | 61.31±1.01 (30.29)* | 68.19±3.21 (22.47)* | 71.54±1.30 (18.66)* |
| | 75 | 87.62±0.64 | 64.54±0.59 (26.34)* | 51.79±0.74 (40.89)* | 46.71±0.69 (46.69)* | 50.72±1.83 (42.11)* | 58.69±0.55 (33.01)* | 65.43±1.43 (25.32)* | 69.15±2.61 (21.07)* |
| Tolbutamide | 40 | 88.44±1.95 | 69.35±1.67 (21.58)* | 52.36±1.21 (40.79)* | 49.13±1.65 (44.44)* | 52.95±1.06 (40.12)* | 63.87±1.45 (27.78)* | 70.52±1.21 (20.26)* | 79.16±0.69 (10.49) |

*All values are expressed as Mean ± SEM, n=5 ; Values given in the parenthesis are percent blood glucose reduction.;*

**Statistically significant P<0.05 compared to 0 h of their respective group.*

***Table 2 - Effects of oral administration of alcoholic extract of E. leucophylla roots on fasting blood glucose level in diabetic rats.***

| Group | Dose (mg/kg) | Blood glucose levels (mg/dl) at different hours | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 h | 2 h | 4 h | 6 h | 8 h | 12 h | 18 h | 24 h |
| Control | ----- | 367.09±2.0 | 359.75±1.4 (1.99) | 341.31±9.7 (7.02) | 339.9±8.6 (7.40) | 333.87±2.5 (9.04) | 328.68±1.1 (1046) | 330.19±4.7 (10.05) | 335.12±8.6 (8.70) |
| *E. leucophylla* | 100 | 337.95±8.9 | 301.54±5.6 (10.77) | 278.68±4.4 (17.53)* | 226.3±4.6 (33.03)* | 249.32±3.5 (26.25)* | 265.2±1.95 (21.52) | 269.98±3.7 (20.11)* | 278.85±7.3 (17.48)* |
| | 250 | 371.38±4.2 | 328.62±5.2 (11.51) | 298.52±2.9 (19.61)* | 216.15±1.1 (41.79)* | 221.32±8.6 (40.40)* | 253.63±1.6 (31.70) | 274.52±2.6 (26.08)* | 285.74±5.1 (23.05)* |
| | 500 | 385.32±2.8 | 332.13±5.3 (13.80)* | 261.61±4.6 (32.10)* | 200.35±6.3 (48.00)* | 213.56±5.4 (44.57)* | 259.61±2.5 (32.62) | 296.31±1.3 (23.10)* | 302.31±2.5 (21.54)* |
| ELR-05 | 25 | 335.31±1.7 | 271.10±1.2 (19.14)* | 230.54±5.7 (31.24)* | 179.52±1.1 (46.46)* | 201.17±0.9 (40.00)* | 262.63±2.1 (21.67) | 275.14±1.3 (17.94)* | 285.12±1.8 (14.96)* |
| | 50 | 367.17±5.3 | 271.52±1.0 (26.05)* | 219.51±2.1 (40.21)* | 180.23±3.1 (50.91)* | 235.45±2.1 (35.87)* | 251.41±1.3 (31.52) | 262.33±8.8 (28.55)* | 274.31±1.5 (25.29)* |
| | 75 | 359.41±4.2 | 268.31±1.8 (25.34)* | 223.51±2.3 (37.81)* | 171.63±3.4 (52.24)* | 198.56±8.5 (44.75)* | 252.54±1.9 (29.730 | 265.18±1.1 (26.21)* | 269.58±2.3 (24.99)* |
| Tolbutamide | 40 | 368.54±3.4 | 289.51±1.6 (21.44)* | 225.75±5.0 (38.74)* | 189.23±1.6 (48.65)* | 222.12±1.6 (39.72)* | 256.67±9.8 (30.35) | 286.45±2.1 (22.27)* | 302.32±5.2 (17.96)* |

*All values are expressed as Mean ± SEM, n=5 ; Values given in the parenthesis are percent blood glucose reduction. ;*
**Statistically significant P<0.05 compared to 0 h of their respective group.*

***Table 3 - Effects of oral administration of alcoholic extract of E. leucophylla roots on fasting blood glucose level in normal and diabetic rats.***

| Treatment | Dose (mg/kg) | Serum insulin (μU/ml) in normal rats | | | | Serum insulin (μU/ml) in diabetic rats | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 h | 4 h | 8 h | 12 h | 0 h | 4 h | 8 h | 12 h |
| Control | ------- | 18.10±0.17 | 16.23±0.35 | 16.12±0.56 | 15.42±2.1 | 5.32±0.51 | 4.91±0.32 | 4.95±0.43 | 5.01±0.52 |
| *E. leucophylla* | 500 | 19.36±0.58 | 32.14±1.2* | 40.13±1.32* | 28.56±0.9 | 4.89±0.56 | 9.31±1.23 | 20.18±1.35* | 16.54±0.98 |
| ELR-05 | 50 | 16.13±1.23 | 34.16±2.3* | 41.13±0.98* | 39.23±1.1* | 4.46±0.23 | 10.31±0.97 | 19.98±0.93* | 18.8±0.67* |
| Tolbutamide | 40 | 16.96±0.75 | 33.45±1.8* | 43.15±1.12* | 36.17±1.3* | 4.57±0.67 | 9.67±0.71 | 21.32±0.54* | 17.6±1.42* |

*All values are expressed as Mean ± SEM. *Statistically significant P<0.05 compared to 0 min of their respective group.*

***Table 4 - Oral glucose tolerance test in normal and experimental animals***

| Groups | Blood glucose levels (mg/dl) | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Normal control | 89.12 ± 2.88 | 172.38 ± 6.02* | 153.72 ± 5.82* | 124.92 ± 3.14* | 95.38 ± 3.28 |
| Normal + *E. leucophylla (500mg/kg)* | 85.12 ± 1.23 | 132.61 ± 4.12* | 113.67 ± 2.31* | 99.58 ±3.17* | 92.67 ±5.12 |
| Normal + ELR-05 | 91.34 ± 3.54 | 128.69 ± 4.13* | 116.52 ±3.71* | 100.75± 1.57* | 92.85 ±1.65 |
| Normal + tolbutamide | 95.63 ± 5.36 | 140.35 ± 1.21* | 111.35 ± 5.85* | 98.34 ±3.45* | 92.17±4.35 |
| Diabetic control | 247.84 ± 4.46 | 322.24 ± 5.47* | 377.01 ± 10.59* | 349.28 ± 3.77* | 316.66 ± 3.57* |
| Diabetic + *E. leucophylla* | 120.64 ± 3.05 | 207.02 ± 5.49* | 183.66 ± 7.66* | 149.06 ± 5.11* | 128.16 ± 3.64 |
| Diabetic + ELR-05 | 135.67 ±5.64 | 199.67 ±6.89* | 175.64 ±5.61* | 141.28 ±3.33 | 116.61±5.52 |
| Diabetic + Tolbutamide | 122.12 ± 1.88 | 207.1 ± 4.62* | 187.34 ± 5.93* | 48.92 ± 4.95* | 129.62 ± 1.92 |

*All values are expressed as Mean ± SEM. *Statistically significant P<0.05 compared to 0 min of their respective group.*

However, it may stimulate insulin secretion like the oral hypoglycemic agents i.e. by influencing depolarization of islet membrane and thereby the changes in the ion-flux, as most of the insulin releasing agents act. Further intensive study can project light on the molecular mechanism of action of insulin secretagogue activity of *Euphorbia leucophylla.*

Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive antidiabetic principles (7, 8, 35, 36). Flavonoids are known to regenerate the damaged beta cells in the alloxan diabetic rats (37). In the present study, a flavone xylopyranoside (compound ELR-05) exhibited dose dependent decrease in blood glucose levels and elevated the serum insulin levels in normal and alloxan induced diabetic rats. Further work is in progress to identify the possible mechanisms of action for its hypoglycemic and antihyperglycemic activities.

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