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# Study of Antidiabetic Polyherbal syrup of Leaf Juice Extracts of Selected Plants

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## ABSTRACT

Polyherbal preparations are presently used for treatment of different diseases. In this study polyherbal syrups of extracts of leaf juice of three selected plants were studied for their antidiabetic potential basing on the use by the tribal people and local vaidyas. *Clerodendrum infortunatum* Linn.(Lamiaceae) has been used against convulsion, diabetes, microbial infections, pain, inflammation. *Paederia foetida* Linn. (Rubiaceae) has been used against rheumatism, diarrhoea, diabetes, cough, liver diseases, pain, ulcer, microbial infections. *Euphorbia neriifolia* Linn. (Euphorbiaceae) has been used against CNS stimulation, abdominal sepsis, liver diseases, diabetes, cancer, inflammation, arthritis and skin diseases etc. In this study, the extracted juice of leaves was subjected to drying and concentrated to get a dry mass. The dry juice mass was fractionated using methanol(70%) after defatting with petroleum ether(60 $^{o}$C – 80 $^{o}$C). Dry mass of juice and Methanol(70%) extract of each plant were investigated. Phytochemical screening revealed that the extracts contain flavonoids, tannins, sugar, saponins, steroids, terpenoids and phenolic compounds. All extracts were investigated for antidiabetic activities. Non sugar syrup was prepared and evaluated. Formulated herbal syrups were studied towards antidiabetic activities on animals after inducing diabetes by alloxan and streptozotocin and compared with the standard drug glibenclamide. Syrup of Methanol(70%) extract at 300mg/kg of each plant showed highest activity than the corresponding dry mass which may be due to presence of high content of phytochemicals. Polyherbal syrup with the Methanol(70%) extract of plant-I, II & III in the proportion 30:40:30 showed better antidiabetic activity.

**KEY TERMS:** Polyherbal, *Clerodendrum infortunatum, Paederia foetida, Euphorbia neriifolia,* leaf juice, Methanol(70%), alloxan, streptozotocin, antidiabetic activity

## INTRODUCTION

After pharmaceutical screening of some plants basing on traditional use by the tribal people and local vaidyas, it was found that the fresh juice of leaves are directly given to patients having diabetes particularly Type-2 diabetes mellitus. Plants are

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available locally which may have different biodiversity with many activities. Considering the knowledge of use since long, *Clerodendrum infortunatum* Linn.(Lamiaceae)[Plant-I], *Paederia foetida* Linn. (Rubiaceae) [Plant-II] and *Euphorbia neriifolia* Linn.(Euphorbiaceae) [Plant-III] were selected for study as herbal syrup against hyperglycemia using the dry mass of fresh juice of leaves & the Methanol(70%) extract of the dry mass. *Clerodendrum infortunatum* Linn. is a shrub seen in many parts of India and other countries.[1] Leaves and their juice are used in inflammation, deworming, vomiting, scabies, fever, helmintic infestation, hyperglycemia and to cure malaria, wound, bone fracture, dysentery, scorpion sting.[2,3] Leaves contain Alkaloids, flavonoids, steroid, saponins, terpenoid, phenolics, glycosides, phytosterols.[4,5] *Paederia foetida* Linn. a climbing plant is native to most of the parts of India, Nepal and other countries.[6] Leaves and their juice are used for the treatment of rheumatism, diarrhea, inflammation, piles, toothache, asthma, bowel problems, diabetes, bacterial infections and seminal weakness.[7,8,9] Leaves yield glycosides, sterol, ursolic acid etc.[10,11] *Euphorbia neriifolia*, is a branched shrub seen in many parts of India including Odisha.[12] Leaves and their juice are used as analgesic, anti-inflammatory, anti-diabetic, antiseptic, antispasmodic and purgative.[13,14] Leaves contain proteins, glycosides, alkaloids, phenolics, flavonoids, saponins, terpenoids, and lignin etc.[15]

The freshly extracted leaf juice of all three plants was studied pharmacologically. The plan was to standardize the syrup of individual extracts. An attempt was taken to prepare suitable polyherbal syrup.

## MATERIAL AND METHOD:

The chemicals used were of analytical grade.

### Collection of plant material:

5 kg of fresh leaves of all plants were gathered separately from different sites of Dukura, Dist- Mayurbhanj, Odisha in March 2020(Fig-1). The plant was authenticated by Dr. R K Sharma, Professor in Botany, Seemanta Mahavidyalaya, Jharpokharia, Dist- Mayurbhanj, Odisha. The specimen was deposited in The Omm Sai Institute of Paramedical Sciences, Dukura, Mayurbhanj, Odisha.

### Extraction and Phytochemical analysis:[16]

Freshly procured leaves were cut in to small pieces and grinded thoroughly and passed through a press/ juicer to collect the juice in bulk. The juice was strained through a fine cloth. The extracted juice was subjected to drying and concentrated in rotary evaporator till complete dryness at 40 $^{o}$C. This extract (dry mass) was subjected for study directly and also fractionated.

The extract (dry mass) was defatted with petroleum ether(60 $^{o}$C – 80 $^{o}$C) and then filtered. The residue was air dried and dispersed in 1000ml of methanol 70%[methanol:water = 70:30] and the flask was shaken by a shaker to disperse the extract and dissolve the soluble components. The flask was kept for 6 hrs on shaking mode and then filtrate was collected, concentrated to get powder. Both the dry mass

of fresh juice and the Methanol(70%) extracts as coded(Table-1) were subjected to phytochemical and pharmacological investigation.

**Table-1. Nomenclature of plants and extracts for study:**

| Plant | Name of plant | Extract for use | Extract Code |
|---|---|---|---|
| I | *Clerodendrum infortunatum* L. | Fresh juice extract dry mass | Ci-F |
| | | Methanol 70% fraction of dry mass | Ci-M |
| II | *Paederia foetida* L. | Fresh juice extract dry mass | Pf-F |
| | | Methanol 70% fraction of dry mass | Pf-M |
| III | *Euphorbia neriifolia* L. | Fresh juice extract dry mass | En-F |
| | | Methanol 70% fraction of dry mass | En-M |

**Phytochemical investigation of extracts:**[17]

Standard procedures were followed for qualitative analysis of phytochemicals present in the extracts. Methods included Molisch test (carbohydrate), Benedict's Test (Reducing sugar), Ninhydrin test(protein), Dragondorff's test(alkaloids), Ferric chloride test(tannins and phenols), General test (glycosides), Alkaline reagent test (flavonoids), Liebermann Burchard test(steroids and terpenoids), Foam test(saponins).

**Determination of Total Flavonoid Content:** [18]

**Method:** Aluminium chloride colorimetric method with a little modification as per the reference was used to determine the total flavonoid content (using standard calibration curve of Quercetin[Fig.2]) which was expressed as weight of quercetin equivalent (QE) at 100g present in the extract of all plants. Observations are recorded in Table-4.

**Determination of Total Phenolic Content:** [19]

**Method**: Folin-Ciocalteau's Reagent (FCR) method was used for quantification of total phenolic content (using standard calibration curve of Gallic acid[Fig.3]) which was expressed as weight of Gallic acid equivalents (GAE) at 100g present in extracts of all plants. Observations are recorded in Table-4.

**Formulation of Non-sugar syrup**:

**Table-2. Formula for Non-sugar syrup:**

| Ingredient | Quantity |
|---|---|
| Sodium carboxymethyl cellulose | 1g |
| Saccharin sodium | 0.1g |
| Benzylkonium Chloride | 0.002g |
| Purified water to | 100ml |

**Method***:* 50 ml of purified water was taken in a beaker. Water was stirred by a high-speed mixer and Sodium carboxymethylcellulose (Na CMC) was slowly added to the vortex formed by the stirrer till dissolved completely forming a clear solution. Saccharin sodium(sweetening agent) and Benzylkonium Chloride (preservative) were added and stirred to dissolve. The prepared solution was transferred to a measuring cylinder and purified water was added to make up the final volume. The prepared

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syrup was filtered through muslin cloth to remove the impurities present in final solution. The solution was transferred to the suitable container for evaluation and use. Benzylkonium Chloride at very low concentration is non toxic. It was added due its stability at alkaline pH of the Na CMC solution.

**Evaluation of Non-sugar syrup**:[20]

The non-medicated syrups were evaluated for various physiochemical parameters and accelerated stability. The syrup was evaluated for following physiochemical parameters such as colour and odour(by human perception), pH( by digital pH meter [ELICO, INDIA]), refractive index(by refractometer[Electronics India]), turbidity(by turbidometer[Electronics India]), viscosity[by Brookfield viscometer] and specific gravity(by using Pycnometer). The syrup at RT and at 40±2 $^0$C, 75±5% humidity was analysed for the period of three months towards stability in terms of colour, odour, pH, specific gravity, refractive index and turbidity/homogeneity.[21] Observations are recorded in Table-5.

**Preparation of medicated syrup:**

Considering the dose of the extracts to be ingested orally (300 mg/kg and 150 mg/kg i.e. 60mg or 30 mg per rat having 200 g body weight approximately), it was decided to incorporate 6% ( i.e. 6g in 100 ml or 60mg/ml) or 3%( i.e. 3g in 100 ml or 30mg/ml) of extract. Each extract was weighed and added to small amount of prepared plain syrup (each type) and stirred well to get a dispersion to which sufficient syrup was then added to get 100 ml.

Glibenclamide was formulated as syrup by dispersing 1.4mg of drug in Non-sugar syrup to make 100ml. Each ml contained 14µg of Glibenclamide. (Adult human dose of drug is 5mg/day)[22]

**Toxicity study:**

In the experiment, healthy Wistar albino rats, about (180-225g, average 200 g.) were maintained in standard conditions (12 hrs. light/12hrs. dark); temperature 25±3 $^0$C and humidity 30-70%. Animals were acclimatized for 4 days prior to the experiment. The research was conducted as per CPCSEA guideline. Initial approval was taken from IAEC of Jeeva Life Sciences, Telengana for animal studies (CPCSEA/IAEC/JLS/18/07/22/028). Both acute toxicity study and sub-chronic (28 days) toxicity study were conducted.

The selected extracts of three plants [Ci-M, Pf-M and En-M] were accurately weighed(5g.each) and suspended in a 1% CMC suspension under mechanical stirring to prepare 100 ml suspension of each extract. Similarly each extract was weighed in equal proportion to get 100 ml of suspension in 1% CMC of poly herbal extracts[PHE].

**Acute toxicity study:**

**Method:** Acute toxicity studies were carried out according to OECD gidelines-423.[23] The prepared suspensions at doses of 0.25/0.5/1.0/3.0g/kg(1/2/4/6ml respectively) were orally fed to rats(n=6). 1% CMC suspension was used as control.

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After oral feeding, the rats were examined for

i) first 3 hour = for any toxic symptoms
ii) up to 7 days daily=for any toxic symptoms, morphological behaviour and mortality.
iii) up to 20 days=to observe mortality

**Sub-chronic (28 days) toxicity study:**

The method of Lewis *et al.*, 2001[24] was followed. The animals were grouped (n=6). Ci-M, Pf-M, En-M and PHE extracts were fed orally once daily at 300 mg/kg to the selected animals for a period of 28 days and the animals were examined twice daily for mortality and morbidity.

**Screening for antidiabetic activity:**

Antidiabetic Study of the extracts of Plant-I [Ci-F & Ci-M], Plant-II [Pf-F & Pf-M] and Plant-III [En-F & En-M] were performed on animals using Wistar albino rats. Following models are followed to evaluate the anti-diabetic activity of different drugs/extracts;

1. Alloxan induced diabetic model
2. Streptozotocin induced diabetic model

**Study protocol:**

**Animals selected**: Wistar albino rats of both sexes of 180-225 g (average 200g) in weight were selected and grouped (n=6) as described below.

| Group | Treatment (mg/kg) | Group | Treatment (mg/kg) |
|---|---|---|---|
| I | Control (1ml syrup) | VIII | Pf-M(150) |
| II | Standard (0.07) | IX | Pf-F(300) |
| III | Ci-F(150) | X | Pf-M(300) |
| IV | Ci-M(150) | XI | En-F(150) |
| V | Ci-F(300) | XII | En-M(150) |
| VI | Ci-M(300) | XIII | En-F(300) |
| VII | Pf-F(150) | XIV | En-M(300) |

**Antidiabetic study by alloxan induced diabetic model:**[25]

**Induction of diabetes**: Wistar albino rats were kept without food for 12 hrs. Freshly prepared alloxan monohydrate solution in the ice cold normal saline (150mg/kg bw) was injected intra-peritoneally to make rats diabetic. Each animal's weight was taken in to account to determine how much alloxan to inject. 72 hours after injection, BGL was determined. Diabetic rats (having BGL> exceeding 220mg/dl) were selected for the study.

**Method**: Diabetic animals were grouped(14x6). Group I animals fed 1ml of non-sugar syrup, Group II animals fed glibenclamide (1ml syrup), and Group III to XIV were fed 1ml each of prepared herbal syrup respectively orally once daily for 12 days. Blood was drawn by puncturing the tail vein with a hypodermic needle, and BGL

were determined on days 0, 1, 3, 6, 9 & 12 days of the therapy. Observations are recorded in Table-6.

**Antidiabetic study by streptozotocin (STZ) induced model:**[26]

**Induction of diabetes**: Wistar albino rats were kept without food for 12 hrs. Freshly prepared STZ solution in the citrate buffer (0.01M, pH-4.5) in ice cold was injected intra-peritoneally(dose- 60mg/kg) to make rats diabetic. 5% glucose was given per oral with water for one day to prevent the chance of lowering BGL. 72 hours after injection, BGL was determined. Diabetic rats (having BGL> exceeding 250mg/dl) were selected for the study.

**Method**: Diabetic animals were grouped(14x6). Group I animals fed 1ml of non-sugar syrup, Group II animals fed glibenclamide (1ml syrup), and Group III to XIV were fed 1ml each of prepared herbal syrup orally once daily for 12 days. Blood was drawn by puncturing the tail vein with a hypodermic needle, and BGL were determined on days 0,1, 3, 6, 9 &12 days of the therapy. Observations are recorded in Table-7.

**Selection of extracts for further study:**

From the antidiabetic study by alloxan and streptozotocin induced diabetic model, it was observed that the Methanol(70%) extract at dose of 300mg/kg of all three plants (in the order Pf-M > Ci-M > En-M) showed significant antidiabetic effect than the dry mass. It was further decided to prepare polyherbal syrup including all these three Methanol(70%) extracts.

**Preparation of polyherbal antidiabetic syrup:**

Four polyherbal syrups were formulated by taking different proportion of the three extacts such as CI-M, PF-M and EN-M all together 6gm/100ml of non-sugar syrup. Methanol(70%) extracts were weighed separately and thoroughly mixed, triturated with few ml of non-sugar syrup and finally diluted with non-sugar syrup to make the volume.

**Table-3. Formulation for polyherbal antidiabetic syrup:**

| Formulation | Formulation code | % of methanol (70%) extracts | | |
|---|---|---|---|---|
| | | Ci-M | Pf-M | En-M |
| 1 | PADS-1 | 30 | 40 | 30 |
| 2 | PADS-2 | 35 | 25 | 40 |
| 3 | PADS -3 | 25 | 40 | 35 |
| 4 | PADS -4 | 40 | 35 | 25 |

**Antidiabetic study of polyherbal syrups by streptozotocin induced model:**

**Method:** The method described previously was followed and BGL were determined on days 0,1, 3, 6, 9 and 12 days of the therapy. Observations are recorded in Table-8 anf Fig.4.

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**Determination of effect of treatment of diabetes on body parameters after 12 days:**

**I. Determination of Body Weight:** Body weight of animals after 12 days treatment were determined

**Method:** Initial body weight and body weight of animals after $12^{th}$ day were measured by electronic balance**.** Observations are recorded in Table-9.

**II. Determination of Biochemical Parameters:**[27,28] Different biochemical parameters were measured after 12 days of treatment as per the method described below and results are noted.

**Estimation of hematological parameters**: Serum insulin, liver glycogen and Glycated haemoglobin were monitored using ELISA and standard kits. Observations are recorded in Table-10.

**Estimation of lipid profiles**: Rats were anaesthetized. Blood was withdrawn from the retro-orbital plexus using capillary(20mm length x 0.8mm diameter) and collected in sample tubes containing EDTA(3mg/ml). Plasma was separated and TC, TG, LDL, HDL were estimated by Auto-Analyzer by standard methods. Observations are recorded in Table-11.

**Estimation of Liver function tests:** Plasma obtained by above procedure was analysed for SGOT, SGPT and ALP content by Auto-Analyzer using the standard methods. Observations are recorded in Table-12.

**Estimation of kidney function tests:** Plasma obtained by above procedure was analysed for urea, uric acid and creatinine using standard diagnostic kits. Creatinine was determined by the alkaline picrate method, urea by the urease-hypochorite method and uric acid by the uricase-peroxidase method. Observations are recorded in Table-13.

## RESULT & DISCUSSION:

**Results of phytochemical investigation***:*

Yield of Ci-F was 10.39% W/V; Pf-F was 9.87%W/V and En-F was 9.12%W/V. These extracts(dry mss) were obtained from the fresh juice of leaves. However the methanol (70%) extract of the dry mass was obtained in high amount due to presence of both methanol and water such as Ci-M[82.23%W/W], Pf-M[85.54%W/W] and En-M[81.72%W/W]. Most of the components were dissolved in the mixed solvent. Both the extracts of each plants contained similar phytoconstituents. However as the weight of Methanol 70% extract obtained was less, it showed these extracts might be having the phytoconstituents in higher proportion on weight basis than the dry mass. The extracts of all three plants contain Glycosides, Flavonoids, Terpenoids, Carbohydrates, Tannins, Proteins, Phenolics. However Plant-III contained alkaloids and saponins while Plant-II contained saponins only. Alkaloids and saponins were absent in Plant-I.

**Results of total Flavonoid & Phenolic content:**

It was observed that the total Flavonoid & Phenolic content of Methanol(70%) extract of Plant I is higher than the Plant II & III. Similar result was obtained in case of dry

mass of juice of leaves. This showed the plants particularly the leaves are rich in flavonoids and phenolic compounds. However due to solvent properties and higher concentration of phytoconstituents in Methanol(70%) extracts, the quantity of phenolic compounds present in these extracts was higher than corresponding dry mass of each leaf juice. The flavonoid content of the dry mass extract of each plant was also comparable to the corresponding Methanol(70%) extracts which may be due to some types of flavonoids present in the dry mass extract which are insoluble in Methanol 70%.

**Results of toxicity studies:**

Acute toxicity study of Ci-M, Pf-M, En-M and PHE revealed that these were non-toxic at 3g/kg. No lethality or toxic reactions were observed till end of study. Hence considering the above safest amount, the therapeutic dose at higher and lower level was selected. 300 mg/kg ($1/10^{th}$ of the dose) and 150 mg/kg were selected as the higher and lower therapeutic dose respectively for further Pharmacological studies.
In sub-chronic (28 days) toxicity study, similar results were obtained as acute study with no mortality and morbidity. , no postural and behavioural changes observed.

**Result of antidiabetic study by alloxan induced model:**

A 12-day course of treatment with multi dose extract syrup was effective in reversing diabetes in rats that had been caused by alloxan. BGL in animals treated with Methanol(70%) syrups containing 300mg/kg decreased significantly ($p< 0.01$) up to 12 days and BGL in animals treated with dry mass of leaves juice syrups containing 300mg/kg decreased significantly ($p< 0.05$) up to 12 days . The other extract syrups with 150mg/kg dose showed non-significant activities.

**Result of antidiabetic study by streptozotocin induced model:**

A 12-day course of treatment with a multi dose extract syrup was effective in reversing diabetes in rats that had been caused by streptozotocin. BGL in animals treated with Methanol(70%) syrups containing 300mg/kg decreased significantly ($p< 0.01$) up to 12 days and BGL in animals treated with dry mass of leaves juice syrups containing 300mg/kg decreased significantly ($p< 0.05/0.01$ ) up to 12 days. The other extract syrups with 150mg/kg dose showed non-significant activities.

**Results of antidiabetic study of polyherbal syrups by streptozotocin induced model:**

BGL of the diabetic animals treated with the polyherbal antidiabetic syrups decreased significantly ($p< 0.01$) up to 12 days. However the formulation PADS-1 showed higher result by decreasing the BGL to the lowest on $12^{th}$ day as compared to other three formulations.

**Result of antidiabetic treatment on body parameters after 12 days:**

**Determination of body weight:** In STZ induced diabetes, the characteristic significant loss of body weight was observed on $12^{th}$ day in positive control group which may be due to an increase in muscle wasting, where as no significant change in

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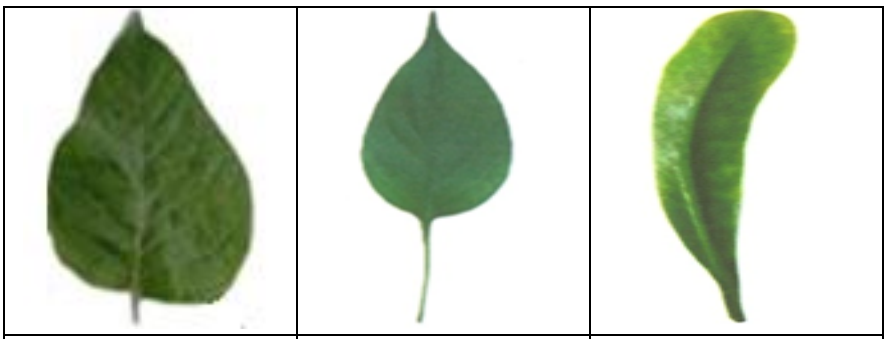
body weight of animals was observed in the groups treated with standard as well as polyherbal antidiabetic syrups.

**Determination of Biochemical parameters:** The values of serum insulin and liver glycogen were increased substantially in STZ control group in comparison to negative control. Animals treated with the standard restored the level. PADS-1 showed significant result ($p<0.01$) at the highest in restoration in comparison to other three polyherbal syrups. Similar activities were seen in case of the level of Glycated Haemoglobin which was increased due to diabetes and restored by use of standard and also PADS-1 followed by other polyherbal syrups.

**Lipid Profile:** The values of cholesterol, triglyceride and LDL were increased substantially in STZ control group in comparison to negative control where as the level of HDL was decreased. Animals treated with the standard restored the levels by $12^{th}$ day of treatment. PADS-1 showed significant result ($p<0.01$) at the highest in restoration in comparison to other three polyherbal syrups.

**Biochemical parameter( Liver function tests):** The values of SGOT, SGPT and ALP were increased substantially in STZ control group in comparison to negative control. Animals treated with the standard restored the levels by $12^{th}$ day of treatment. PADS-1 showed significant result ($p<0.01$) at the highest in restoration in comparison to other three polyherbal syrups.

**Biochemical parameter( Kidney function tests):** The values of Serum urea, creatinine and Uric acid were increased substantially in STZ control group in comparison to negative control. Animals treated with the standard restored the levels by $12^{th}$ day of treatment. PADS-1 showed significant result ($p<0.01$) at the highest in restoration in comparison to other three polyherbal syrups.



| *Clerodendrum infortunatum* L. | *Paederia foetida* L. | *Euphorbia neriifolia* L. |
|---|---|---|

**Fig 1. Leaves of *selected plants***

**Table-4. Total Flavonoid and Phenolic content of extracts of plants:**

| Plant | Extract | Total flavonoids (mg/100gm) as QE | Total phenolics (mg/100gm) as GAE |
|---|---|---|---|
| I | Ci-F | 100.86±4.67 | 96.27±3.24 |
| | Ci-M | 101.02±2.81 | 118.36±2.67 |

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| | | | |
|---|---|---|---|
| II | Pf-F | 97.71±3.28 | 94.23±3.43 |
| | Pf-M | 98.53±4.06 | 116.10±3.02 |
| III | En-F | 92.76±3.84 | 88.63±3.18 |
| | En-M | 93.57±2.48 | 110.41±4.13 |

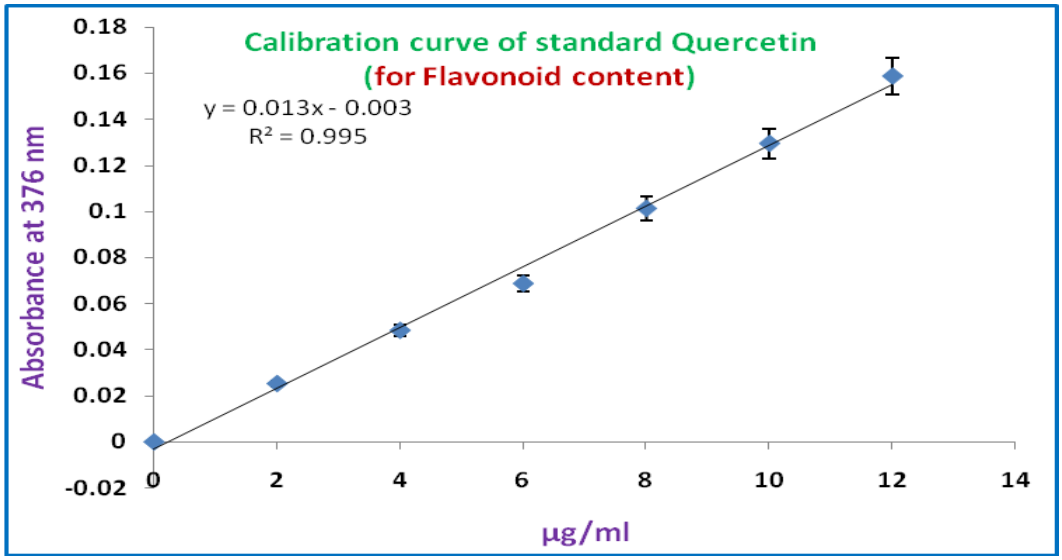


**Fig. 2. Calibration curve of standard quercetin.**

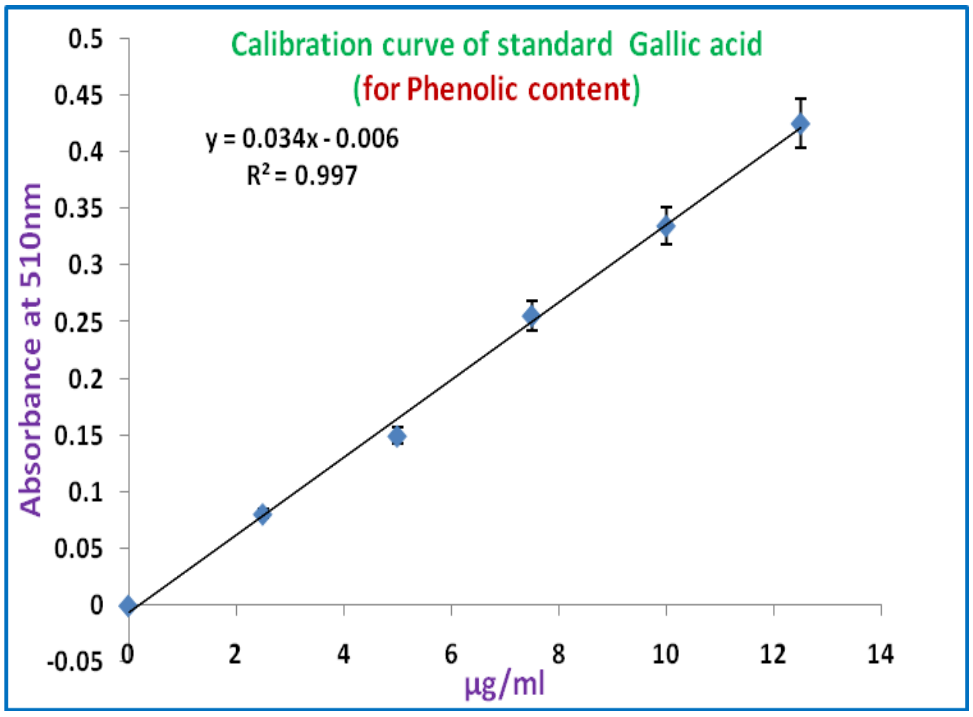


**Fig.3. Calibration curve of standard gallic acid.**

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**Table-5. Observations of different parameters(Initial and after accelerated studies) of Non sugar syrup:**

| Parameters | Observation | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | Accelerated studies | | | | | |
| | | 1st month | | 2nd Month | | 3rd Month | |
| | | RT | 40$^0$C | RT | 40$^0$C | RT | 40$^0$C |
| Colour | Colour less | Colour less | Colour less | Colour less | Colour less | Colour less | Colour less |
| Odour | Pleasant | Pleasant | Pleasant | Pleasant | Pleasant | Pleasant | Pleasant |
| Turbidity | Clear | Clear | Clear | Clear | Clear | Clear | Clear |
| Specific gravity | 1.0072 | 1.0071 | 1.0069 | 1.0071 | 1.0070 | 1.0070 | 1.0069 |
| pH | 7.3 | 7.3 | 7.2 | 7.3 | 7.1 | 7.2 | 7.1 |
| Refractive index | 1.3341 | 1.3332 | 1.3311 | 1.3291 | 1.3299 | 1.3301 | 1.3392 |
| Viscosity in cps | 1520 | 1520 | 1516 | 1520 | 1515 | 1520 | 1514 |

**Table-6. Effect of extract syrups on BGL in alloxan-induced diabetic rats:**

| Groups | Treatment (mg/kg) | BGL(mg/dL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0day | 1day | 3day | 6day | 9day | 12day |
| I | Control (1ml syrup) | 95.45 ±3.54 | 97.25 ±3.42 | 94.46 ±3.75 | 95.75 ±2.34 | 94.86 ±2.76 | 93.87 ±4.03 |
| IA | Alloxan control | 225.45 ±3.54 | 232.17 ±4.24 | 219.36 ±5.38 | 202.41 ±6.37 | 193.78 ±3.87 | 184.41 ±6.37 |
| II | Standard (0.07) | 228.74 ±4.26 | 185.12 ±5.25 | 143.43 ±4.21$^*$ | 119.38 ±5.37$^*$ | 79.53 ±4.86$^{**}$ | 71.38 ±5.37$^{**}$ |
| III | Ci-F(150) | 222.22 ±2.75 | 228.47 ±4.23 | 217.52 ±5.37 | 194.31 ±4.24 | 184.04 ±3.87 | 174.13 ±5.03 |
| IV | Ci-M(150) | 227.42 ±3.64 | 231.21 ±2.85 | 216.47 ±5.17 | 194.15 ±5.68 | 183.87 ±4.75 | 173.46 ±4.89 |
| V | Ci-F(300) | 225.37 ±2.85 | 208.53 ±2.31 | 189.47 ±2.98 | 154.25 ±3.54 | 121.58 ±4.75$^*$ | 95.12 ±5.72$^*$ |
| VI | Ci-M(300) | 223.75 ±3.81 | 198.21 ±5.39 | 176.35 ±5.42 | 137.78 ±4.75$^*$ | 85.54 ±5.10$^{**}$ | 81.53 ±4.21$^{**}$ |
| VII | Pf-F(150) | 227.65 ±4.12 | 227.23 ±5.23 | 214.27 ±4.68 | 192.54 ±4.76 | 181.38 ±3.83 | 172.42 ±5.38 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| VIII | Pf-M(150) | 228.35 ±1.43 | 225.42 ±4.21 | 213.76 ±4.38 | 190.21 ±5.31 | 180.39 ±4.67 | 170.53 ±4.76 |
| IX | Pf-F(300) | 223.85 ±3.12 | 211.52 ±5.02 | 191.98 ±4.23 | 146.23 ±5.16 | 117.74 ±6.05* | 93.03 ±3.51* |
| X | Pf-M(300) | 225.43 ±4.05 | 203.53 ±6.35 | 173.44 ±4.18 | 135.13 ±3.86* | 83.02 ±4.06** | 79.42 ±3.76** |
| XI | En-F(150) | 227.21 ±2.63 | 228.07 ±2.87 | 216.05 ±3.65 | 193.27 ±4.21 | 182.52 ±6.04 | 173.18 ±4.86 |
| XII | En-M(150) | 224.57 ±1.87 | 227.23 ±2.38 | 215.65 ±1.87 | 192.42 ±5.86 | 181.75 ±3.45 | 172.32 ±5.21 |
| XIII | En-F(300) | 225.56 ±4.39 | 217.36 ±4.85 | 180.31 ±3.74 | 159.42 ±4.21 | 123.74 ±5.13* | 97.09 ±4.38* |
| XIV | En-M(300) | 222.35 ±3.27 | 192.65 ±3.54 | 170.14 ±2.68 | 121.67 ±3.41* | 91.28 ±4.38* | 83.43 ±4.23** |

*Values are expressed as mean ± SEM; n=6; Statistical significance vs. control (* $p<0.05$, ** $p<0.01$ ); One way ANOVA followed by Student's t-test.*

**Table-7. Effect of extract syrups on BGL in streptozotocin-induced diabetic rats:**

| Groups | Treatment (mg/kg) | BGL(mg/dL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0day | 1day | 3day | 6day | 9day | 12day |
| I | Control (1ml syrup) | 93.21 ±2.36 | 95.22 ±2.21 | 96.34 ±1.75 | 94.52 ±2.12 | 95.32 ±1.03 | 94.03 ±2.03 |
| IA | STZ control | 264.14 ±4.32 | 260.52 ±3.35 | 244.75 ±2.85 | 219.32 ±5.63 | 201.52 ±4.51 | 194.05 ±5.38 |
| II | Standard (0.07) | 269.21 ±4.32 | 239.43 ±5.02 | 201.24 ±4.75 | 147.53 ±4.85* | 85.38 ±3.53** | 70.75 ±4.86** |
| III | Ci-F(150) | 268.45 ±4.74 | 261.12 ±3.23 | 236.24 ±4.25 | 209.43 ±4.75 | 194.30 ±3.12 | 178.34 ±4.78 |
| IV | Ci-M(150) | 274.35 ±5.28 | 265.01 ±4.74 | 235.14 ±4.25 | 207.74 ±5.37 | 190.13 ±4.82 | 176.15 ±3.56 |
| V | Ci-F(300) | 270.25 ±3.56 | 245.37 ±2.87 | 207.74 ±3.65 | 155.43 ±4.21* | 100.24 ±3.57* | 89.42 ±5.10** |
| VI | Ci-M(300) | 271.54 ±3.84 | 243.41 ±4.23 | 204.12 ±5.10 | 152.72 ±6.21* | 98.27 ±5.36** | 81.67 ±5.23** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| VII | Pf-F(150) | 264.47 ±4.42 | 258.75 ±4.23 | 234.55 ±4.65 | 206.51 ±4.78 | 189.15 ±4.87 | 174.53 ±3.75 |
| VIII | Pf-M(150) | 272.76 ±2.83 | 259.57 ±4.45 | 232.78 ±4.68 | 205.85 ±5.21 | 188.59 ±3.85 | 173.24 ±4.32 |
| IX | Pf-F(300) | 268.65 ±3.46 | 249.97 ±5.32 | 205.74 ±4.88 | 154.41* ±5.46 | 100.16 ±5.21* | 88.75 ±5.11** |
| X | Pf-M(300) | 271.53 ±4.87 | 242.62 ±5.37 | 203.25 ±5.28 | 149.54 ±3.86* | 96.42 ±3.69** | 80.38 ±3.74** |
| XI | En-F(150) | 279.41 ±3.63 | 265.23 ±4.75 | 236.30 ±4.18 | 207.02 ±4.25 | 193.25 ±4.87 | 176.53 ±5.31 |
| XII | En-M(150) | 269.10 ±2.57 | 257.54 ±4.66 | 234.41 ±5.04 | 206.15 ±4.38 | 190.56 ±4.77 | 175.75 ±6.45 |
| XIII | En-F(300) | 275.43 ±4.03 | 252.62 ±4.05 | 210.23 ±4.74 | 158.30 ±4.53* | 102.07 ±5.74* | 90.56 ±4.54** |
| XIV | En-M(300) | 278.37 ±4.21 | 250.75 ±4.54 | 208.54 ±4.61 | 156.41 ±2.98* | 100.12 ±3.68* | 83.74 ±3.56** |

*Values are expressed as mean ± SEM; n=6; Statistical significance vs. control ( * $p<0.05$, ** $p<0.01$ ); One way ANOVA followed by Student's t-test.*

**Table-8. Effect of polyherbal antidiabetic syrups on BGL in streptozotocin-induced diabetic rats:**

| Groups | Treatment (mg/kg) | BGL(mg/dL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0day | 1day | 3day | 6day | 9day | 12day |
| I | Control (1ml syrup) | 90.35 ±3.36 | 91.46 ±2.54 | 92.74 ±3.54 | 91.24 ±4.10 | 91.21 ±2.56 | 90.53 ±3.87 |
| IA | STZ control | 276.45 ±3.54 | 263.12 ±4.21 | 262.23 ±3.57 | 226.42 ±5.62 | 221.57 ±5.34 | 205.36 ±3.68 |
| II | Standard (0.07) | 272.74 ±5.21 | 234.35 ±4.21 | 192.68 ±5.42* | 139.43 ±4.64* | 86.74 ±5.01** | 69.25 ±3.86** |
| III | PADS-1 | 275.56 ±5.42 | 238.24 ±3.85 | 196.52 ±4.74* | 144.20 ±3.68* | 90.01 ±4.96** | 74.29 ±3.56** |
| IV | PADS-2 | 272.34 ±4.86 | 242.15 ±4.35 | 201.53 ±2.87* | 148.31 ±4.21* | 93.54 ±3.54** | 79.15 ±3.56** |
| V | PADS -3 | 276.86 ±5.10 | 240.74 ±4.86 | 199.78 ±2.85* | 147.85 ±3.74* | 91.21 ±5.21** | 78.38 ±3.74** |
| VI | PADS -4 | 275.64 ±4.38 | 239.35 ±6.35 | 198.65 ±6.28* | 147.24 ±5.86* | 91.38 ±4.10** | 78.05 ±5.23** |

*Values are expressed as mean ± SEM; n=6; Statistical significance vs. control ( ** $p<0.01$ ); One way ANOVA followed by Student's t-test.*

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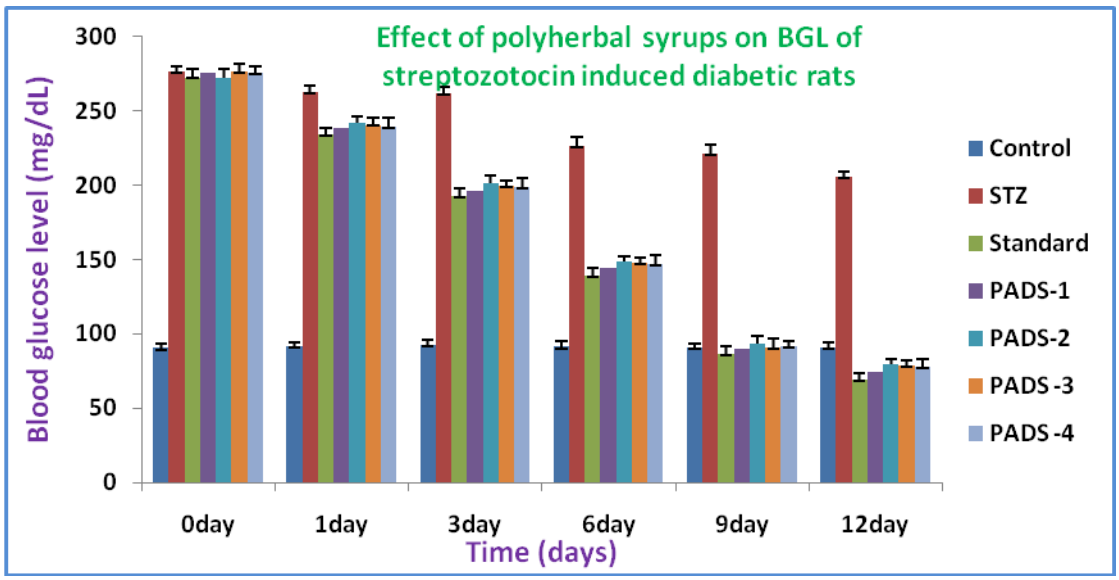


**Fig.4. Effect of polyherbal antidiabetic syrups on BGL in streptozotocin-induced diabetic rats.**

**Table-9. Effect of treatment with polyherbal antidiabetic syrups on change in body weight in diabetic rats:**

| Groups | Treatment (mg/kg) | Body weight(g) | |
|---|---|---|---|
| | | Day 0 | Day 12 |
| I | Control (1ml syrup) | 205.41±6.78 | 204.27±7.45 |
| IA | STZ control | 199.45±8.21 | 182.11±7.78 |
| II | Standard (0.07) | 201.43±9.24 | 198.37±8.63 |
| III | PADS-1 | 204.36±9.36 | 197.36±8.36 |
| IV | PADS-2 | 203.76±10.04 | 198.78±12.25 |
| V | PADS -3 | 200.84±8.13 | 195.55±9.37 |
| VI | PADS -4 | 203.14±6.19 | 194.41±6.14 |

*Values are expressed as mean±SD(n=6)*

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**Table-10. Effect of treatment with polyherbal antidiabetic syrups on Haematological parameters:**

| Groups | Treatment (mg/kg) | **Serum Insulin (µU/ml)** | **Gly Hb(%)** | **Liver Glycogen(mg/g)** |
|---|---|---|---|---|
| I | Control (1ml syrup) | 13.34±3.12 | 3.90±0.16 | 8.63±0.21 |
| IA | STZ control | 6.34±0.57 | 8.91±0.34 | 5.75±0.32 |
| II | Standard (0.07) | 13.84±0.73** | 3.75±0.45** | 8.91±0.28** |
| III | PADS-1 | 12.38±1.03** | 4.11±0.37** | 7.91±0.62** |
| IV | PADS-2 | 11.97±0.56* | 4.23±1.56* | 7.45±0.54* |
| V | PADS -3 | 11.68±0.78* | 4.36±0.85* | 7.23±0.75* |
| VI | PADS -4 | 11.43±0.63* | 4.58±0.07* | 7.01±0.53* |

*Values are expressed as mean±SD(n=6). Statistical significance vs.+ve control ( * p<0.05, ** p<0.01 ); Oneway ANOVA followed by Students t test.*

**Table-11. Effect of treatment with polyherbal antidiabetic syrups on lipid profiles:**

| Groups | Treatment (mg/kg) | **Cholesterol (mg/dL)** | **Triglycerides (mg/dL)** | **LDL (mg/dL)** | **HDL (mg/dL)** |
|---|---|---|---|---|---|
| I | Control (1ml syrup) | 109.21± 2.35 | 90± 1.34 | 64± 1.68 | 49± 0.46 |
| IA | STZ control | 217.42± 2.73 | 159.37± 0.98 | 141.03± 1.25 | 19.54± 0.87 |
| II | Standard (0.07) | 97.39± 2.14** | 82.34± 1.65** | 50.24± 0.75** | 47.36± 0.53** |
| III | PADS-1 | 111.52± 1.65* | 89.41± 1.32** | 62.03± 1.03** | 50.22± 1.25** |
| IV | PADS-2 | 114.35± 1.32* | 92.21± 1.89** | 64.40± 2.03** | 53.57± 0.67** |
| V | PADS -3 | 117.15± 1.28* | 94.08± 1.74** | 67.01± 1.07** | 53.00± 1.42** |
| VI | PADS -4 | 120.24± 1.57* | 97.61± 2.21** | 70.03± 1.87** | 57.58± 1.53** |

*Values are expressed as mean±SD(n=6). Statistical significance vs.+ve control ( * p<0.05, ** p<0.01 ); Oneway ANOVA followed by Students t test.*

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**Table-12. Effect of treatment with polyherbal antidiabetic syrups on biochemical parameter( Liver function tests):**

| Groups | Treatment (mg/kg) | SGOT (U/L) | SGPT (U/L) | ALP (U/L) |
|---|---|---|---|---|
| I | Control (1ml syrup) | 33.73±1.15 | 24.23±0.69 | 133.13±4.43 |
| IA | STZ control | 75.47 ± 1.19 | 55.35 ± 3.74 | 185.35 ± 2.54 |
| II | Standard (0.07) | 30.14± 1.37** | 21.85± 0.68** | 136.34 ± 3.25* |
| III | PADS-1 | 32.45 ± 0.52** | 25.76 ± 1.45* | 137.14 ± 2.45* |
| IV | PADS-2 | 37.63 ± 0.89* | 28.24 ± 1.32* | 141.27 ± 1.85* |
| V | PADS -3 | 38.52 ± 1.56* | 29.13 ±0.87* | 142.36 ± 2.35* |
| VI | PADS -4 | 41.74 ± 1.86* | 31.75 ±1.39* | 140.15 ± 3.58* |

*Values are expressed as mean±SD(n=6). Statistical significance vs.+ve control ( * p<0.05, ** p<0.01 ); Oneway ANOVA followed by Students t test.*

**Table-13. Effect of treatment with polyherbal antidiabetic syrups on biochemical parameter( Kidney Function Test):**

| Groups | Treatment (mg/kg) | Serum urea (mg/dL) | Serum creatinine (mg/dL) | Uric acid (mg/dL) |
|---|---|---|---|---|
| I | Control (1ml syrup) | 28.20±0.25 | 0.93±0.05 | 1.78± 0.45 |
| IA | STZ control | 79.42 ± 1.84 | 1.43 ± 0.14 | 4.58 ± 0.68 |
| II | Standard (0.07) | 27.63 ± 0.69** | 0.91 ± 0.08** | 1.77 ± 0.44** |
| III | PADS-1 | 30.12 ± 1.53** | 0.95 ± 0.38** | 1.89 ± 0.56** |
| IV | PADS-2 | 32.52 ± 1.02** | 1.01 ± 0.12* | 2.11 ± 0.31* |
| V | PADS -3 | 34.43 ± 1.78 ** | 1.03 ± 0.09* | 2.23 ± 0.75* |
| VI | PADS -4 | 34.75 ± 0.89 ** | 1.01 ± 0.06* | 2.35 ± 0.426* |

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*Values are expressed as mean±SD(n=6). Statistical significance vs.+ve control ( $^{*}$ $p<0.05$, $^{**}$ $p<0.01$ ); Oneway ANOVA followed by Students t test.*

## CONCLUSION

*Clerodendrum infortunatum* Linn, *Paederia foetida* Linn. and *Euphorbia neriifolia* Linn. are well known plants. Different parts are medicinally used in different forms. Local vaidyas use the fresh juice of leaves of these selected plants. Many researchers evaluated the pharmacological activity of the plant. However, from the literature survey, it was found that no much study has been done scientifically on the antidiabetic potential of the fresh juice of leaves of the plants particularly available in the collected area considering their biodiversity. The fresh juice was dried to get dry mass for study. Methanol(70%) extract was prepared from dry mass. The extracts were rich in phytoconstituents such as flavanoids, phenols, tannins, glycosides and sterols etc. Extracts were studied for total flavonoid and phenolic contents. It was observed that the extracts were rich in both flavonoid and phenolic. The extracts were studied for toxicity and found safe upto the dose of 3000mg/kg on rat. Hence the extracts were studied at 300mg/kg as higher dose and 150mg/kg at lower dose. Syrup of the extract was prepared using non-sugar syrup containing Na CMC, Saccharin. Study of antidiabetic potential of the extracts were conducted using alloxan and streptozotocin induced diabetic models. In both the study it was observed that the syrups containing Methanol(70%) extract at a dose of 300mg/kg showed significant decrease in BGL($p<0.01$) in comparison to the dry mass($p<0.05$ or $p<0.01$). The extracts with 150mg/kg dose showed non-significant effect. Considering the reduction in BGL on $12^{th}$ day by the extracts which was observed in the order Pf-M > Ci-M > En-M, the polyherbal syrups were prepared. While studying the antidiabetic activity, it was observed that, PADS-1 showed higher result by decreasing the BGL to the lowest on $12^{th}$ day as compared to other three formulations. But all the polyherbal formulations showed significant result in lowering BGL($p<0.01$). All the polyherbal formulations showed profound result in restoring the various haematological and biochemical parameters which were either increased or decreased towards better result due to diabetes. The result concluded that the Methanol(70%) extract of leaves of all selected plants can be used effectively as antidiabetic and can be formulated as polyherbal non-sugar syrup. If the syrups are properly formulated and studied in a scientific manner including clinical studies, the formulation can be used therapeutically.

**Statistical analysis:**

All the data were presented as mean± SEM. The statistical differences were evaluated by ANOVA, followed by Student *t*-test. Statistical significance on comparison with the control were considered by $p<0.001$ highly significant, $p<0.01$ more significant and $p<0.05$ significant.

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**ABBREVIATIONS**

ALP - Alkaline phosphatase
BGL - Blood glucose level
Ci - *Clerodendrum infortunatum* L.
En - *Euphorbia nerifolia* L.
F - Fresh juice dry mass
GAE - Gallic acid equivalents
HDL - High density lipid
Hrs - Hours
LDL - Low density lipid
M - Methanol(70%) extract
Pf - *Paederia foetida* L.
PHE - Poly Herbal Extract
QE - Quercetin equivalent
RT - Room Temperature
SGOT - Serum glutamic-oxaloacetic transaminase
SGPT - Serum glutamic-pyruvic transaminase
TC - Total cholesterol
TG - Triglyceride

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