

EVALUATION OF IN-VIVO ANTIDIABETIC ACTIVITY OF POLYHERBAL EXTRACT

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ABSTRACT

The main aim of the work is to determine the anti-diabetic activity of mixture of hydroalcoholic extracts of *Phyllanthus emblica* (Fruits), *Annona reticulata* (leaves), *Cesalpinia bonducella* (seeds) were collected from local area, dried, pulverized and extracted with ethanol and water (7:3). Anti-diabetic effect of extract was studied by using metformin as a standard on alloxan induced diabetic rats. There were six groups of animal in that I was normal, II was Control, III was standard. There were a three combination of mixture of extracts were administered to group IV, V, VI after that blood sugar levels were measured at 0, 2, 4, 8, 16, 24, 32 hrs respectively by taking blood at retro orbital route with the help of accu check active glucometer. **Results:** Significant lowering of blood sugar was observed after administration of combination I (*Phyllanthus emblica* (100mg), *Annona reticulata* (100mg), *Cesalpinia bonducella* (100mg) of mixture of extracts **Conclusion:** combination of mixture of *Phyllanthus emblica* (Fruits), *Annona reticulata* (leaves), *Cesalpinia bonducella* (seeds) shows significant antidiabetic activity.

Keywords: *Phyllanthus emblica*, *Annona reticulata*, *Cesalpinia bonducella*, Metformin, Alloxan induced diabetic rats.

1. INTRODUCTION

1.1 Diabetes

There are more than 125 million people with diabetes in the world today and this number is expected to approach 220 million. It is also estimated that there are 30 to 33 million diabetics in India now, and every fourth diabetics in the world today is an Indian. Indians are genetically more susceptible to diabetes and the WHO predicts the number of diabetes in India would group to 80 million by 2030 [1]

1.1 Diabetes Mellitus:

It is a clinical syndrome characterized by hyperglycemia due to the pancreas may produce little insulin, if any. In other cases, the pancreas may produce some insulin, but the cells do not respond to it. Diabetes mellitus is characterized by hyperglycemia, glucosuria, negative nitrogen balance and sometimes ketonemia [2] In diabetic people blood sugar remains high due to neither is insulin in body nor insulin is insufficient, or is not as effective as it should be. [3]

Diabetes mellitus is classified as follows

Type I: Immune mediated - Juvenile onset /Insulin dependent (could be in children with a more rapid onset or adults with a slower onset late autoimmune diabetes of adults),

Type II: Insulin resistant -Adult onset diabetes/non-insulin dependent diabetes

A. Gestational diabetes mellitus

B. Other specific types (e.g. certain genetic defects; drug induced; etc). [4]

Type I is most common form of diabetes(5%) and it is autoimmune disorder, and type 2(95%) it is associated with the obesity .Whereas gestational diabetes is occurs in pregnancy ,other forms of diabetes are very rare and which are caused due to single gene mutation . [5] According to World Health Organization about 80T% population relies on traditional medicine [6].There are many herbal plants which are used to treat the disease .they contain bioactive compound which is responsible to cure the certain disease. [7]

1.2 Introduction to herbal plants

1.2.1 *Phyllanthus emblica* (Amla):

Phyllanthus emblica is reported to treat many more disease . Amla is used to treat the diabetes .Fresh amla juice help to increase glow of skin. [2] The fruit has one of the highest concentrations of Vitamin C (160 times more than apple) and thus is reputed as a strong antioxidant. Amla finds uses in many medicinal and cosmetic products, especially those for hair such as hair oils and tonics. Amla fruit is used in Indian cooking mainly as pickles or as mouth-freshners. It is also a constituent of the Dabur Chawanprash. [8, 9]

1.2.3 *Caesalpinia boducella* (Sagar gota):

Seeds of *Caesalpinia boducella* are used as an antiperiodic, antirheumatic ,antidiabetic. Leaves and bark are used as an anthelmintic . Root of these plants are diuretic,and anticalculous. Leaves and twigs are traditionally used for the treatment of tumors, inflammation and liver disorder. They have also been applied for treatment of toothache. Leaves and juices have been used traditionally for elephantiasis and smallpox. [8] The seed is claimed to be styptic, purgative and anthelmintic and cures inflammations, useful in colic, malaria, hydrocele, skin diseases and leprosy. The seeds are considered tonic, ferifuge, anthelmintic, antibleorrhagic, Seed and long pepper powders taken with honey gives good expectorant effect. [9] The seeds are ground in water and given internally in snake-bite. The kernel of the seed is very useful and valuable in all ordinary cases of simple, continued and intermittent fevers. Seed kernels show antidiabetic potential. [10] Bark of root possesses number of properties like febrifuge, intestinal worms, amenorrhoea, cough, and anthelmintic etc. In Jamaica, it is used as rubifacient and as a local application for sores. [11]

1.2.4 *Annona reticulata* (Ramphala)

Annona reticulata leaves antidiabetic .leaves are employed in tanning and also yield a blue or black dye The leaves are used internally against worms, and externally to treat abscesses A concentrated extract of the see is employed to remedy dysentery and diarrhea. Fragments of the root bark are put around the gums to relieve toothache. The root decoction is taken as a febrifuge. The seeds, leaves and young fruits have insecticidal properties The plant is poisonous and has a potential as a pesticide for non-vertebrates.[8, 9]

2. Material and method

2.1 Collection And Procurement:

All three plants were collected from Ahmednagar district (Maharashtra state ,India).Leaves of *Annona reticulata* ,fruits of *Phyllanthus emblica* and seeds of *Caesalpinia bonducella* were shade dried.The dried parts were cleaned and coarsely powdered in grinder and powder material was passed through 120 mesh to remove fine powder and coarse powder was used for extraction.

2.2 Authentication:

All three plants were authenticated by Priyanka Ingale, Scientist B of Botanical Survey of India, Pune, through comparing morphological features. The herbarium of the plant specimen was deposited at Botanical Survey of India, Pune .Voucher specimen numbers are as follows ,

Table.1 Voucher specimen number of plants authentication

Sr. No.	Name Of Plants	Voucher Number
1	<i>Ceasalpinia bonducella</i> (Ceasalpiniaceae)	RT01
2	<i>Annona reticulata</i> (Annonaceae)	RT02
3	<i>Phyllanthus emblica</i> (Phyllanthaceae)	RT03

2.3 Extraction:

2.3.1Extraction of *Phyllanthus emblica*:

The fruits of *Phyllanthus emblica* were collected and shade dried ,then pulverized in electric grinder .About 100 gms of powdered fruits were used for extraction, powder were passed through 120 mesh sieve to remove fine powder and coarse powder and coarse powder was used for extraction.[12]

Solvent used for extraction: Alcohol and water

Technique: Reflux

50 gms of dried powder were extracted with 7:3 mixture of alcohol and water by using reflux for 6 hrs .After complete extraction filtrate were collected and air evapourated .Extract were stored in air tight container in refrigerator until use. [13]

2.3.2Extraction of *Annona reticulata*:

The leaves of *Annona reticulata* were collected and shade dried ,then pulverized in electric grinder .About 100 gms of powdered leaves were used for extraction ,powder were passed through 120 mesh sieve to remove fine powder and coarse powder and coarse powder was used for extraction.[14]

Solvent used for extraction: Petroleum ether, Alcohol and water

Technique :soxhlet extraction.

The powdered leaves of *Annona reticulata* were extracted with petroleum ether (60-80) for removal of colouring matter by defatting processs using continuous soxhlet extraction method.After complete defatting the defatted powder were reflux with alcohol and water (7:3)for 5 hrs. Extraction temperature was maintained at 50°C .The extract was filtered and concentrated to get thick paste and after it freeze dried to get powder .The extract was stored in air tight container (Mukherjee, 2002). [15]

2.3.3 Extraction of *Ceasalpinia bonducella*:

The seeds of *Ceasalpinia bonducella* were collected and shade dried ,then pulverized in electric grinder .About 100 gms of powdered seed kernels were used for extraction, powder were passed through 120 mesh sieve to remove fine powder and coarse powder and coarse powder was used for extraction.[16]

Solvent used for extraction: Petroleum ether, Alcohol and water

Technique: soxhlet extraction and reflux

The powdered leaves of *Ceasalpinia bonducella* were extracted with petroleum ether (60-80) for removal of colouring matter by defattating processs using continuous soxhlet extraction method.After complete defatting the defatted powder were reflux with alcohol and water (7:3)for 5 hrs .extraction temperature was maintained at 50°C.

The extract was filtered and concentrated to get thick paste and after it freeze dried to get powder .The extract was stored in air tight container (Mukherjee 2002). [17]

3. Preliminary phytochemical screening for various extracts:

A phytochemical screening of carbohydrates ,proteins ,an]mino acids alkaloids ,tannin flavonoids saponin terpenoids ,sterols,vitamins present in extracts was performed by using standard method .[19]

4. Pharmacological Activity

4.1 Experimental animals:

Wistar strain albino rats weighing between 150-180 gm were obtained from Lacsmi Biofarms private limited,Pune . The rats were housed in cleaned metallic cages and kept in well ventilated room and allowed to acclimatized to the laboratory condition for one week before being used. They were fed with standard animal pellet and had free access to water and libitum.The animal were randomly divided into six groups .The protocol of the experiment (1942/PO/Re/S/17/CPCSEA/2018/01) was approved by Institutional Animal Ethics Committee (IAEC) of Pravara Rural college of pharmacy ,Loni and were conducted in accordance with permission from committee for the purpose of control and supervision of Experiments on Animals (CPCSEA) . [20]

4.2 Acute toxicity study of Extract (LD50)

Sr.No.	Name of groups	Treatment
1	Normal control	Normal saline injection
2	Diabetic control	Normal saline injection
3	Standard	Metformin 600 mg/kg of body weight
4	Test (Combination I)	Extract 300mg /kg of body weight
5	Test (Combination II)	Extract 300mg /kg of body weight
6	Test (Combination III)	Extract 300mg /kg of body weight

Various combinations of the given three extract were prepared in following manner

Table 2 Combinations of Extract

Sr. No.	Name of Extract	Combination I	Combination II	Combination III
1	<i>Phyllanthus emblica</i>	100 mg	125 mg	75 mg
2	<i>Annona reticulata</i>	100 mg	75 mg	100 mg
3	<i>Ceasalpinia bonducella</i>	100 mg	100 mg	125 mg

4.6 Analysis of blood sugar levels:

Blood samples were collected by retro-orbital plexus after overnight fast at the intervals of 0, 2, 4, 8, 16, and 32 hrs. The blood glucose level in the samples was estimated using Accucheck Active Glucometer . [24]

5. Result:

5.1 Preliminary phytochemical screening for various extracts :

Preliminary phytochemical screening for various extracts shows presence of following phytoconstituents:

Extracts	<i>Phyllanthus emblica</i>	<i>Annona reticulata</i>	<i>Ceasalpinia bonducella</i>
Test for Carbohydrates			
a. Molisch Test	+	+	+
b. Fehling Test	+	+	+
c. Benedict Test	+	+	+
Test for Proteins			
a. Biuret Test	-	-	-
b. Million's Test	-	-	-
Test for Steroids			
a. Salkowski Test	+	-	+
b. Liebermann- Burchard reaction	+	-	+
c. Liebermann reaction	+	-	+
Test for Glycosides			
a. Borntrager's Test	-	-	-
b. Keller-Killiani Test	-	-	-

Test for Saponin			
a. Foam Test	-	-	+
Test for Flavonoids			
a. Shinoda Test	+	+	+
b. Lead acetate Test	+	+	+
Test for Alkaloids			
a. Dragendroff's Test	+	+	+
b. Mayer's Test	+	+	+
c. Hager's Test	+	+	+
d. Wagner's Test	+	+	+
Test for Amino acids			
a. Ninhydrin Test	-	-	-
b. Test for Tyrosine	-	-	-
c. Test for Tryptophan	-	-	-
d. Test for Cysteine	-	-	-
Test for Tannins and phenolic compound			
a. 5% FeCl ₃ solution	+	-	-
b. Lead acetate solution	+	-	-
c. Bromine water	+	-	-
d. Acetic acid	+	-	-
Test for Vitamins			
a. Test for Vitamin C	+	-	-

(+ indicates presence of phytoconstituents, - Indicates absence of phytoconstituents)

The hydroalcoholic extract of *Phyllanthus emblica* (fruits) *Annona reticulata* (leaves), and *Cesalpinia bonducella* (seeds) were combined together at 300 mg/kg dose .three combinations were made in that I combination all three extracts were same that was each 100 mg/kg ,in second combination *Phyllanthus emblica*(125 mg),*Annona reticulata*(75mg) ,and *Cesalpinia bonducella* (100mg) and in third combination *Phyllanthus emblica*(75 mg),*Annona reticulata*(100mg) ,and *Cesalpinia bonducella* (125mg) respectively . All three combinations were

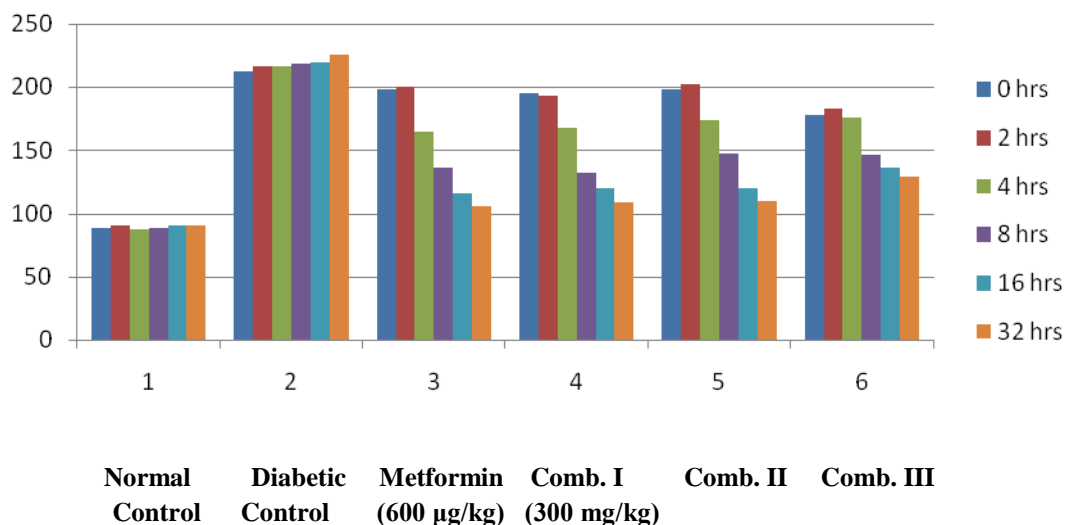
administrate to group IV, V VI number and lowering of blood sugar was determined by using accu-check active glucometer at 0, 2, 4, 8, 16, 32 hrs respectively.

5.2. Estimation of blood glucose level:

Table 15 Effect of various polyherbal combinations on alloxan induced diabetic rats

Treatment	Blood Sugar Level(mg/dL)					
	0 hr	2 hr	4hrs	8 hrs	16 hrs	32hrs
Control	87.92±3.84	90.02±2.43	87.46±1.21	88.90±2.56	90.36±2.76	90.41±3.64
Diabetic control	212.9±8.24	216±7.58	216±8.64	219±9.51	219.07±9.64	225.8±8.92
Metformin	198±11.26	200±12.45	165±11.89	136±13.12	116±12.86	105.8±11.65
Combination I	195±5.68	193±7.56	168±7.69	132±6.74	120±5.23	108.8±8.00*
Combination II	198±6.73	202±5.00	174±3.7	147±8.9	120.4±11.43	109.7±14.78*
Combination III	177.7±11.45	183±9.83	176±11.78	146.3±14.26	136±11.3	129.4± 1.23*

*P <0.05 when compared with control (no drug) animal. Values are given as mean ±SEM for 6 rats in each group.



Graph.1 Changes on blood glucose level in groups of normal and alloxan induced diabetic rats

6. Discussion:

In above three combination of polyherbal extract combination I means *Phyllanthus emblica*(100 mg),*Annona reticulata*(100mg) ,and *Caesalpinia bonducella* (100mg)shows significant lowering of blood glucose level as compared to other two combination.

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