

# Antioxidant and Antidiabetic Activity of Phyllanthus Emblica Fruit Extract in Wistar Rats

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**Abstract:** The present study was performed to assess the antioxidant and antidiabetic activity of Phyllanthus emblica fruit extract. The antioxidant activity was carried out in different concentrations of aqueous fruit extract using DPPH assay. The aqueous fruit extract of Phyllanthus emblica exhibited more scavenging activity of DPPH in a dose-dependent manner when compared to the control. The antidiabetic activity of Phyllanthus emblica fruit extract was studied in male wistar rats. The rats were divided into 5 groups. Group I served as the control group. Group II was rendered diabetic through intraperitoneal injection of streptozotocin (60mg/Kg body weight). Group III received aqueous fruit extract of Phyllanthus emblica (200mg/kg body weight) daily through oral gavage for 28 days. Group IV rats received glibenclamide (600µg/Kg body weight) daily through oral gavage for 28 days and group V received only the aqueous fruit extract of Phyllanthus emblica. The study revealed a significant increase in the blood glucose levels and a decrease in the plasma insulin levels in the diabetic rats when compared to the control rats indicating the antidiabetic activity of Phyllanthus emblica fruit extract. The study suggests that Phyllanthus emblica fruit extract can be recommended for use as a natural supplementary herbal remedy to the diabetic patients.

**Keywords:** Phyllanthus emblica, Rats, antioxidant, antidiabetic.

## Introduction:

An antioxidant is a bioactive compound which scavenges the free radicals from the body cells thus preventing or reducing the damage caused due to reactive oxygen species (ROS) and which are produced during mitochondrial oxidative metabolism as well as in cellular response to xenobiotics, cytokines and bacterial invasion. The free radicals are generated as a consequence of a number of endogenous metabolic exposure to several exogenous chemicals [1]. The antioxidants may mediate their effect either by directly reacting with ROS, quenching them or by chelating the metal ions [2].

Diabetes is the most common metabolic disorder affecting the people of both developed and underdeveloped countries. It is considered as a crucial public health challenge [3]. Apart from insulin, several synthetic oral hypoglycemic drugs are available for treating diabetes [4] but possess side effects [5,6]. However, in spite of a number of antidiabetic medicines available in the pharmaceutical market, herbal remedies from different plant sources are used with success to treat diabetes since they are considered to be less toxic, free from side effects with relatively low costs [7]. In addition several medicinal plants are rich source of bioactive secondary metabolites with strong pharmacological actions [8]. These plants are prescribed to treat diabetes since they possess numerous phytoconstituents such as alkaloids, carotenoids, flavonoids, glycosides, saponins and terpenoids having antidiabetic activities [9]. The combined or synergistic activity of the biologically active compounds present in the medicinal plants may contribute to the possible beneficial action [8]. The antidiabetic effect resulting from the treatment with these medicinal plants may be due to their potential to boost the efficacy of pancreatic tissue by increasing insulin secretions or decreasing glucose absorption [9].

Phyllanthus emblica is a traditional medicinal tree found in most of the tropical and subtropical countries particularly in central and southern India, Bangladesh, Pakistan, Srilanka, Mascarene Islands, Southern China as well as Malaysia [10, 11]. It is a medium sized tree bearing fruits and having a height of 8-10 metres with thin light grey bark and small thin irregular flakes [12]. The various parts of Phyllanthus emblica that are mostly used include flowers, fresh and dried fruits, leaves and root bark [13]. The flowering and fruiting season of this plant are February – May and December-January respectively [10]. Since several decades, the fruits of Phyllanthus emblica with their sour or bitter, sweet, cooling, rejuvenative, astringent and antipyretic characteristics were useful as traditional medicine. Further, the fruit of Phyllanthus emblica is broadly used in traditional system of medicine such as Indian Ayurvedic medicine, Chinese herbal medicine and Tibetan medicine [14]. Several previous studies reported that Phyllanthus emblica had good health effects as antioxidant, antidiabetic, antimicrobial, anti-inflammatory as well as hepatoprotective agent [15, 16].

The present study was performed to study the antioxidant and antidiabetic activity of aqueous fruit extract of Phyllanthus emblica in wistar rats.

## Materials and Methods:

All the chemicals used were of Analytical grade.

### Preparation of Ethanolic fruit extract of Phyllanthus emblica:

The fruits of Phyllanthus emblica were collected from the local market, deseeded, washed, dried and then powdered. About 100 gms of powder was extracted using 200ml of 95% ethanol for 72 hours. Then the suspension was filtered with muslin cloth and the extract was concentrated using a rotary evaporator at 45°C under reduced pressure. Later the filtrates were collected in the evaporating dish and placed on the water bath to solidify the extract. About 2 mg of the extract was dissolved in 10ml of ethanol

forming a stock solution with a concentration of 200µg/ml. Different concentrations of the extract ranging between 20 to 100 µg/ml were prepared by diluting the stock solution with ethanol.

**Antioxidant Activity:** The antioxidant activity of ethanolic fruit extract of *Phyllanthus emblica* was determined using DPPH method[17]with slight modifications.

#### DPPH Assay:

DPPH assay is based on the ability of DPPH, a stable free radical to decolourise in the presence of an antioxidant. It is considered as a direct and reliable method for determining the radical scavenging action of a chemical.

Five different concentrations of ethanolic fruit extract of *Phyllanthus emblica* ranging from 20 to 100µg/ml were placed in individual cuvettes and about 3ml of 0.1mM methanolic solution of DPPH radical was added. The mixture was then vigorously shaken and allowed to stand for 30 minutes in the dark at room temperature. The control (Ascorbic acid) contained all the reaction reagents except the ethanolic fruit extract and methanol was used for baseline correction. The absorbance was measured at 517nm using a spectrophotometer and the results were compared with the standard oxidants. The ability of DPPH scavenging activity was then calculated using the formula

DPPH Scavenging activity (% Inhibition)=  $\frac{AO-A1}{AO} \times 100$

Where AO is the absorbance of the control and A1 is the absorbance of the test sample (ethanolic fruit extract of *Phyllanthus emblica*).

#### Antidiabetic Activity:

##### Induction of Diabetes:

Healthy male wistar rats weighing between 150 to 200 gms were used for the experiment. Diabetes was induced in a group of overnight fasted rats by administration of a single intraperitoneal (IP) injection of freshly prepared Streptozotocin (STZ) dissolved in 0.1 M citrate buffer (P<sup>H</sup> 4.5) with a dose of 60mg/kg/body weight. The STZ treated rats were allowed to drink 10% glucose solution overnight to overcome the drug induced hypoglycemia. After 48 hours of STZ administration, induction of diabetes was verified by measuring the blood glucose level. The animals having a blood glucose level ranging above 200-300mg/dl were considered as diabetic and used for the experiment.

**Experimental Design:** Group I rats received normal saline and served as the control group. Group II included the diabetic rats. Group III included the diabetic rats treated with ethanolic fruit extract of *Phyllanthus emblica* (200mg/kg body weight) daily through oral gavage for 28 days. Group IV included the diabetic rats treated with glibenclamide(600µg/kg body weight) in aqueous solution daily through oral gavage for 28 days. Group V included the control rats treated with ethanolic fruit extract of *Phyllanthus emblica* (200mg/kg body weight) daily through oral gavage for 28 days.

The body weights of the rats was monitored and were measured before and after the experiment. At the end of the study i.e. after 28days, all the rats were sacrificed by cervical decapitation. The blood samples were withdrawn from all the rats through retro-orbital plexus puncture and used for the estimation of glucose. The plasma was separated for the estimation of insulin. The blood glucose was estimated by O-toluidine method[18].

**Statistical Analysis:** Statistical analysis was performed using one way Analysis (ANOVA). All the results were expressed as Mean ± S.D and P-value < 0.05 were considered to be significant.

**Results and Discussion:-** The effect of different concentrations of ethanolic fruit extract of *Phyllanthus emblica* is shown in Table-I and Figure-I. In the present study, the ethanolic fruit extract of *Phyllanthus emblica* exhibited more scavenging activity of DPPH in a dose- dependent manner when compared to control (Ascorbic acid). Similar high scavenging activity of DPPH were reported in *Phyllanthus emblica* extract by Yadav et al[19] and Sumalatha [20] which bears a testimony to our present findings. *Phyllanthus emblica* possess antioxidant activity and is mainly responsible for the cytoprotective action in non-steroidal anti-inflammatory drug induced ulcer as suggested by Ananya Chatterjee et al[21]. Further, Rehman et al [22] advocated that the antioxidant effect of the plant products (*Phyllanthus emblica*) is mainly due to the radical scavenging activity of the phenolic compounds such as flavonoids, polyphenols, tannins as well as phenolic terpenes.

**Table -I**

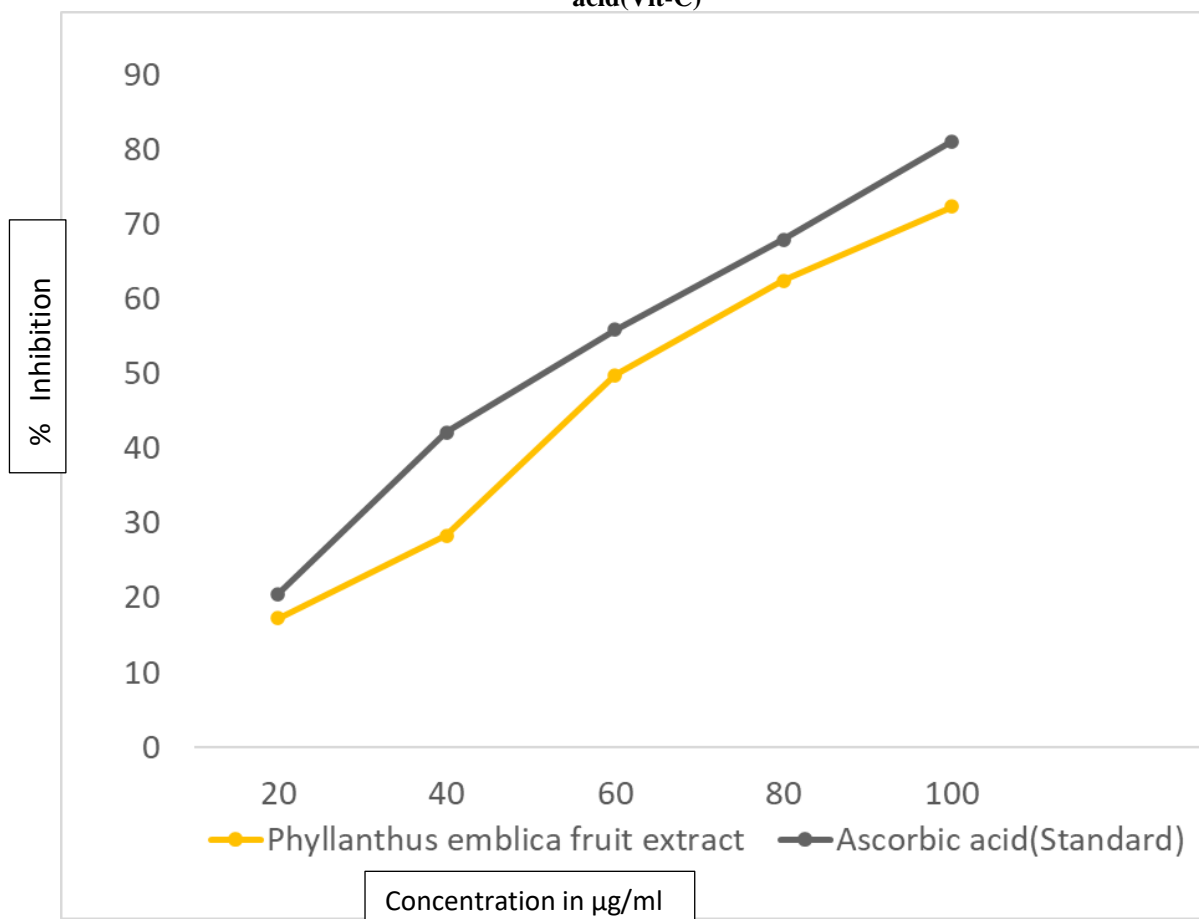
**DPPH Radical scavenging activity of different concentrations of *Phyllanthus emblica* fruit extract and Ascorbic acid(Vit-C)**

Sample	Concentration in µg/ml	DPPH Scavenging activity (% Inhibition)
Phyllanthus emblica fruit extract	20	17.28±0.79*
	40	28.36±0.30*
	60	49.88±1.21*
	80	62.49±0.60*
	100	72.43±1.08*

Ascorbic acid(control)	20	20.48±0.69
	40	42.16±2.12
	60	55.89±2.25
	80	67.99±0.32
	100	81.20±1.10

Values are presented as Mean±S.D . \*P<0.05 compared to control.

**Figure-1 DPPH Radical Scavenging activity of different concentrations of Phyllanthus emblica fruit extract and Ascorbic acid(Vit-C)**



The body weight of the control and treated rats are summarized in Table-II. The study revealed that the body weight of the diabetic rats were significantly decreased when compared to that of the control rats. Supplementation of Phyllanthus fruit extract and glibenclamide to the diabetic rats showed a significant improvement in the body weight of the diabetic rats. However, no significant changes were observed between the control (group I) and control treated group (group V). Table-III summarizes the blood glucose and plasma insulin levels in control and treated rats. The present study revealed a significant increase in the blood glucose levels and a decrease in the plasma insulin levels in the diabetic rats when compared to the control rats. Administration of Phyllanthus emblica and glibenclamide to the diabetic rats elevated the blood glucose levels. However, no variation was observed in blood glucose level between the control (group I) and control treated rats (group V). The plasma insulin levels showed a decline in all the treated groups (group III to V) compared with the control (group I).

**Table-II**  
**Effect of Phyllanthus emblica on Body weight of Rats**

Group	Initial Body weight(gm) (0 <sup>th</sup> Day)	Final Body weight(gm) (28 <sup>th</sup> Day)
I-Normal control	176.14±5.60	212.76±8.08 *
II-Diabetic control	185.06±4.59	148.05±6.47 *
III- Diabetic+ Phyllanthus emblica	187.20±3.21	172.24±7.43 *
IV-Diabetic+ glibenclamide	189.04±5.13	176.44±9.06 *
V-Control+Phyllanthus emblica	180.41±6.53	202.54±6.70 *

Values are expressed as Mean±S.D (n=6 Rats);\* p<0.05 compared to control.

**Table-III**  
**Effect of Phyllanthus emblica on Blood Glucose and Plasma Insulin levels in Rats**

Group	Blood Glucose (mg/dL)	Plasma Insulin(μU/ml)
I-Normal control	80.62±7.02	15.69±1.40
II-Diabetic control	267.85±15.42*	6.89±0.56*
III-Diabetic+ Phyllanthus emblica	121.70±8.02*	13.82±0.51*
IV-Diabetic+glibenclamide	91.46±7.59	14.80±0.69
V-Control+Phyllanthus emblica	81.08±4.80	15.60±0.58

Values are presented as Mean±S.D (n=6 Rats);\*p< 0.05 compared to control.

Streptozotocin (STZ) is an antibiotic that is produced by streptomyces achromogenes, a gram positive bacterium [23]. It is used as a chemotherapeutic agent to treat pancreatic β-cell carcinoma and also to induce diabetes in experimental rat model. It damages the insulin producing beta cells of the pancreas in mammals. It interferes with cellular metabolic oxidative mechanism as reported by Papaccio et al[24]. The streptozotocin (STZ) induced diabetes is characterized by severe loss in body weight of diabetic rats which could be due to degradation and catabolism of fats and proteins [25]. Further increased catabolic reactions leading to muscle wasting may be the cause for weight loss in the diabetic rats as suggested by Raj kumar, et al[26]. In the present study, the administration of ethanolic fruit extract of Phyllanthus emblica and glibenclamide normalized the body weight of the diabetic rats suggesting the protective effect of the extract in controlling muscle wasting in glycolysis. The fruits of Phyllanthus emblica is claimed to be useful in controlling diabetes which are concomitant with our present findings. The results indicate that the Phyllanthus fruit extract was found to reduce the blood glucose level in STZ induced diabetic rats accompanied by a significant decrease in plasma insulin levels when compared with control rats. The observations of the present study are in complete agreement with the reports by several workers stating that STZ-induced diabetes mellitus and insulin deficiency leads to increased blood glucose[27-29]. This may be due to the result of impairment of peripheral tissues of the liver to metabolize glucose.

Phytochemical analysis of the Phyllanthus emblica fruit as reported by several workers revealed the presence of tannins, flavonoids, alkaloids, terpenoids, carbohydrates as well as proteins. These compounds are considered as powerful antioxidants to scavenge the free radicals induced by hyperglycemia. Herbal extracts from the medicinal plants containing flavonoids and tannins were reported to demonstrate the antibiotic activity[30]. On the basis of the above evidence as reported by several workers, it is possible that the flavonoids and tannins present in the Phyllanthus fruit extract may be responsible for the observed antidiabetic activity.

**Conclusion:** Our present study shows that the ethanolic fruit extract of Phyllanthus emblica possess the antioxidant as well as antidiabetic effect in streptozotocin induced diabetic rats. It shows a therapeutic promise as a protective agent against several possible related cardiovascular complications in diabetes mellitus. It has considerable potential for improving the public health if consumed on regular basis. However, further pharmacological as well as biochemical investigations are needed to find out the active constituents responsible for the antidiabetic activity and to elucidate its mechanism of action.

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