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Evaluation of Antioxidant and Antidiabetic Activity of *Phyllanthus emblica* (Fruit)

ASIFA BASHIR[1], AAMIR MUSHTAQ[2*] & TOOBA MEHBOOB[3]

[1]Riphah Institute of Pharmaceutical Science, Riphah International University Lahore
[2]Gulab Devi Institute of Pharmacy, Gulab Devi Educational Complex, Ferozpur Road Lahore
[3]Faculty of Pharmacy, University of Central Punjab, Lahore

ABSTRACT

The objective of this study was to explore the scientific basis of traditional usage of *Phyllanthus emblica* as an anti-diabetic agent in hyperglycemic patients. Ethanolic extract of its dried fruits was analyzed both qualitatively and quantitatively for evaluation of phytochemical agents. Then its antioxidant and anti-diabetic activities were found. The results indicated the presence of carbohydrates, tannins, phenols, alkaloids, flavonoids, saponins, glycosides, amino acids and proteins in crude extract with phenolic and flavonoid contents observed as 9.9 mg/g equivalent of gallic acid and 100 mg/g equivalent of rutin, respectively. Free radical scavenging activity by plant extract was observed as 68±0.33 % as compared to rutin (58±1.15 %) at concentration 2.56 mg/ml. Similarly, *in vivo* studies indicated that *Phyllanthus emblica* 80 mg/kg significantly reduced the glucose level in diabetic rats up to 166 ± 0.7 mg/dl on day 8^{th} at 8^{th} h as compared to diabetic rats 380 ± 0.7 mg/dl. Similarly, it also reduced the weight in rats up to 274.11 ± 0.97 g as compared to 310.71 ± 0.57 g which was recorded before treatment. It was thus concluded that *Phyllanthus emblica* is a potent antioxidant and antidiabetic agent.

Key words: *Phyllanthus emblica,* antidiabetic, Amla, diabetes mellitus, antioxidant

INTRODUCTION

The prevalence of diabetes mellitus is increasing day by day which needs to be controlled on priority basis. Trend is shifting towards the use of herbal remedies so various plants (*Pterocarpus marsupium, Momordica charantia* and *Trigonella foenum-graecum* etc) have been scientifically studied for their anti-diabetic effect (Jung *et al.*, 2006).

Medicinal plants endure to be of an essential therapeutic benefitfor relieving disorders of mankind. From the previous 2500 years, a variety of traditional medical systems including Ayurvedic, Unani and Chinese are in practice. These traditional medicinal systems have been created and largely practiced in the eastern countries. On the whole, nearly 80% population of the developing countries largely depend upon these systems of traditional medicine for their basic health needs (Tsay & Agrawal, 2005).

Herbal preparations and plants are widely used for the treatment and management of diabetes mellitus all over the world instead of the availability of modern glucose lowering agents. The current research was performed to investigate the hypoglycaemic activity of the fruit extract of *Phyllanthus emblica* in insulin resistance diabetic rat models and the effect of plant extract on the fasting glucose level was also found.

Phyllanthus emblica (Euphorbiaceae) is commonly known as Amla and generally grows in subtropical as well as tropical areas in China, India, Asia, Indonesia and also on the Malay Peninsula. All parts of plant like roots, stem, leaves and fruits are used both in fresh and dry forms (Unander *et al.*, 1990).

It has been increasingly used in Ayurvedic system of medicines as a diuretic, laxative and coolant. In food stuffs, it is used to make jellies and pickles (Shankar *et al.*, 1996). It possesses strong antioxidant activities (Naik *et al.*, 2005). Traditionally, it has been used as anti-inflammatory, antiulcer, anti-cancer, hepatoprotective and immune-modulating agent (Dang *et al.*, 2011).

Chemical studies have shown that Amla is enriched in vitamin-C (Nisha *et al.*, 2004) and gallic acid. It also contains ellagic acid, gallic acid, corilagin, furosin, flavonoids, glycosides and tannins which are responsible for anti-inflammatory, anti-microbial, anti-ulcer, nephroprotective, analgesic and antipyretic, anti-tussive, and immune modulating activities (Dang *et al.*, 2011; Kumaran & Karunakaran, 2006; Zhang *et al.*, 2001).

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***Corresponding author**: aamir_mushtaq@hotmail.com

MATERIALS AND METHODS

Plant Material and Extraction

The Amla (*Phyllanthus emblica*) fruits were obtained from the local market of Lahore, Pakistanand duly authenticated by Department of Botany, University of the Punjab, Lahore. They were then dried in the shade and carefully grinded to a coarse sizepowder by electrical grinder and then the powder was passed through 24 mesh size sieve. The powder of Amla fruit (500 g) was then allowed to dip in 70% aqueous ethanolsolution for three days with occasional stirring and mixture was drained first with muslin cloth and after that it was strained by Whatmann filter paper in order to achieve the extract in the fine form. The filtrate was then concentrated by using rotary evaporator and finally a thick viscous paste of dark brown color was obtained.Itwas thenweighed to obtain percentage yield of the extract and extract was then stored in an air lockcontainer and was appropriately labeled. Container was placed in a refrigerator at low $4^{O}C$.

Phytochemical analysis

Many kinds of secondary metabolites or constituents like tannins, alkaloids, phenols, flavonoids, nitrogen compounds, vitamins, terpenoids, steroids present in plant extract wereanalyzed by using simple qualitative tests (Mushtaq *et al.*, 2013).

Quantitative analysis of phenols in plant extract

Folin-Ciocalteu reagent method Kumaran & Karunakaran, (2007)with some modifications was used toassess the quantity of phenol in the aqueous crude extract of *Phyllanthus emblica*. In 1ml of plantextract 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% solution $Na2CO_3$ were added.The resulting mixture was allowed to incubate for 15 minutes at room temperature .The sampleabsorbance was determined at 765nm. Gallic acid was used as standard in concentration of 1mg/ml.The tests were performed in triplicates and results of the test were determined by thestandard curve and were mentioned as gallic acid equivalent (mg/g of extracted compound).Total phenols = Gallic acid Eq. (µg/mL) × extract (vol.) / sample (g)

Quantitative analysis of flavonoids in plant extract

To determine the total flavonoid content, a method by (Kumaran & Karunakaran, 2007)was used. Toa 10 ml test tube, 0.3 ml of extract, 3.4 ml of 30% methanol, 0.15 ml of $NaNO_2$ (0.5 M) and 0.15 ml of $AlCl_3.6H_2O$(0.3 M aluminum chloride) were combined and mixed. 1 ml of NaOH (1 M) solution was added after 5 mins. The resulting solution was mixed well and the absorbance of the solution was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was made by using rutin as standard solution (0 to 100 mg/l). The total flavonoidswere expressed as milligrams of rutin equivalents per g of dried fraction.

Antioxidant activity

Plant extract was dissolved in DMSO and stock solution was also prepared. The stock solution (1mg/ml) was taken to prepare different concentration. To measure the free radicalscavenging activity DPPH method was used. Different concentrations of the crude extract(0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 and 2.56 mg/ml) were prepared in DMSO and a solution of 0.01 mM of DPPH in DMSO was also prepared. 1mL of DPPH solution was addedin each different concentration. The control was also prepared by addition of DPPH solution in3mL of DMSO. The test tubes were kept in darkness for 30 min at room temperature. Absorbance was measured at 517nm against blank. Different concentrations of Rutin was also prepared which was used as a standard(Blois, 1958). Each sample was tested three times. Graph was plotted between% scavenging activity and concentration by which the value of IC50 was calculated. DPPH radical scavenging activity was measured by following formula.% age scavenging = [Absorbance of control – Absorbance of sample/ Absorbance of control] x 100.

Animals

Male albino rats weighing 200-300 g were used in this study which were procured and housed in the animal house of Riphah Institute of Pharmaceutical Sciences, Riphah International University Lahore. The rats were kept under standardroom temperature of 25±2 ˚C and a relative humidity of 45-55% and supplied with a light and dark cycle of 12:12 hr. The rats were placed in a hygienic environment during the entire study Before initiating the study the animals were acclimatized at least for one week of work. The experimental study design was provided as per the ethical considerations for animal management and handling. All the animals had free access to water and they were fed with commercially available rat food pellet.

Study Design

Total of 36 rats were equally divided into six groups in such a way that G-1 was administered with normal food and water. G-II was diabetic control group administered with dexamethasone 10 mg/kg/i.p once daily. G-III to VI were administered with dexamethasone 10 mg/kg/i.p + glimiperide 20 mg/kg/i.p, dexamethasone 10 mg/kg/i.p+ *Phyllanthus emblica* 40 mg/kg/i.p, dexamethasone 10 mg/kg/i.p+ *Phyllanthus emblica* 60 mg/kg/i.p and dexamethasone 10 mg/kg/i.p+ *Phyllanthus emblica* 80 mg/kg/i.p, respectively for eight days. Hyperglycemia was induced in all the groups except normal control group by administration of dexamethasone 10 mg/kg/i.p daily for seven days and then later on animals were treated according to the protocols of study design for eight consecutive days and blood glucose was measured at 0 hr, 2 hr, 4 hr and 8 hr after dosing of animals. Blood was drawn from tail of rat and glucose level was measured by using glucometer. Similarly, weight of animals before and after the treatment was noted for each animal.

Statistics Tools

All the statistical data /results were assessed by using Graph Pad Prism 6. TWO WAYANOVA was applied and the values /data were presented in tabular form as a Mean ± standarderror of mean. The results were summarized by comparing the experimentalgroups to disease groups and the normal group. The level of significance was shown in term of P-value ($P \leq 0.05$)

RESULTS

Phytochemical Analysis

Qualitative phytochemical analysis revealed the presence of carbohydrates, tannins, phenols, alkaloids, flavonoids, saponins, glycosides, amino acids and proteins in crude extract of *Phyllanthus emblica* as shown in Table I.

Total Phenols and Flavonoids

Total phenolic and flavonoids contents of ethanolic extracts of *Phylanthus emblica* were recorded as 9.9 mg/g equivalent of gallic acid and 100 mg/g equivalent of rutin, respectively.

Free Radical Scavenging Activity

The percentage of DPPH free radical scavenging activity of the ethanolic extracts of *Phyllanthus emblica* indicated that by increasing the concentration, scavenging activity was also increased against Rutin. As IC_{50} is increased, scavenging activity is decreased and vice versa.

Effect of Ethanolic Extract of *Phyllanthus Emblica* on Blood Glucose Level

It was observed that high blood glucose levels were recorded in disease controlled animals in which diabetes was induced as compared to normal control group. The blood glucose level was observed to be decreased significantly in animals treated with ethanolic extract of *Phyllanthus emblica* and the reference drug glimepiride when compared with the disease control. Moreover, it was observed that on day 1^{st} and 2^{nd}, least reduction in glucose levels was observed at various time intervals. But the effect of different doses of plant extract was clearly observed on day 4^{th}, 6^{th} and 8^{th} as shown in Table III.

Table I: Phytochemical analysis of *Phyllanthus emblica*

Chemical Constituents	Tests	Presence
Protein and amino acids	i. Millon's test ii. Ninhydrin	+++ ++
Carbohydrates	i. Fehling Test ii. Benedict test iii. Molisch's Test iv. Iodine Test	- ++ ++ +
Tannins and phenols	i. Iodine test ii. Ferric chloride test iii. Nitric acid test	- - +
Flavonoids	i. Alkaline Reagent ii. Lead acetate	++ ++
Saponins	Foam Test	++
Glycosides	i. Liebermann's Test ii. Salkowski's Test iii. Keller-kilani Test	- ++ +
Steroids	Test for Steroids	++
Alkaloids	i. Hager's Test ii. Wagner Test iii. Mayer's Test iv. Dragendroff's Test	++ +++ ++ ++
Terpenoids	Test for Terpenoids	-

+ sign indicates slightly present, ++ indicates moderately present, +++ represents highly present and – sign indicates absence

Table II: DPPH free radical scavenging activity of ethanolic extract of *Phyllanthus emblica* and Rutin

Sr. No.	Concentration mg/ml	% age scavenging by Rutin (%)	% age scavenging by *Phyllanthus emblica* (%)
1	0.02	14±0.66	58±1.155*
2	0.04	17±1.14	60±1.155*
3	0.08	18±1.16	61±1.155*
4	0.16	35±1.12	65±1.155*
5	0.32	40±1.11	66±1.155*
6	0.64	43±1.21	68±0.577*
7	1.28	48±1.24	67±1.155*
8	2.56	58±1.15	68±0.33*

Each Value was expressed as Mean ± SEM. n=3.*P<0.05 was given as compared to the value of Rutin

Table III: Effect of ethanolic extract of *Phyllanthus emblica* on blood glucose level at various time intervals

Groups	Blood Glucose Levels (mg/dl) on Day 1st				Blood Glucose Levels (mg/dl) on Day 2nd				Blood Glucose Levels (mg/dl) on Day 4th				Blood Glucose Levels (mg/dl) on Day 6th				Blood Glucose Levels (mg/dl) on Day 8th			
	0 h	2 h	4 h	8h	0 h	2 h	4 h	8h	0 h	2 h	4 h	8h	0 h	2 h	4 h	8h	0 h	2 h	4 h	8h
G-I	99 ± 0.5	100 ± 0.5	101 ± 0.5	99 ± 0.9	99 ± 0.5	99 ± 0.7	100 ± 0.5	98 ± 0.5	99 ± 0.5	100 ± 0.5	99 ± 0.5	100 ± 0.5	98 ± 0.5	103 ± 0.6	104 ± 0.5	99.6 ± 0.4	101 ± 0.5	99 ± 0.7	99.33 ± 0.4	101 ± 0.8
G-II	263 ± 0.9	268 ± 0.5	271 ± 0.5	283 ± 0.7	285 ± 0.7	289 ± 0.7	293 ± 0.7	301 ± 0.5	315 ± 0.7	318 ± 0.5	321 ± 0.5	328 ± 0.5	341 ± 1.3	342 ± 1.2	346.3 ± 0.4	355 ± 1.1	367 ± 0.5	369 ± 0.7	373 ± 0.5	380 ± 0.7
G-III	281 ± 0.5*	278 ± 0.5*	271 ± 0.5	263 ± 0.5*	285 ± 0.5	281 ± 0.7*	278 ± 0.5*	267 ± 0.5*	248 ± 0.5*	246 ± 0.5*	240 ± 0.5*	232 ± 0.5*	215 ± 0.5*	209 ± 0.4*	207.1 ± 0.6*	210 ± 1.7*	178 ± 0.5*	171 ± 0.7*	163 ± 0.5*	155 ± 1.1*
G-IV	298 ± 0.5*	291 ± 0.6*	287 ± 0.7*	278 ± 0.7*	283 ± 0.5	278 ± 0.7*	271 ± 0.7*	261 ± 0.7*	260 ± 0.5*	257 ± 0.5*	250 ± 0.5*	237 ± 0.5*	221 ± 1.1*	218 ± 0.3*	216.6 ± 0.7*	207 ± 0.5*	189 ± 0.7*	181 ± 1.6*	178 ± 0.7*	171 ± 0.7*
G-V	299 ± 0.7*	295 ± 0.6*	291 ± 0.5*	283 ± 0.5	301 ± 0.7*	298 ± 0.5*	291 ± 0.7	275 ± 0.5*	251 ± 0.5*	248 ± 0.5*	241 ± 0.9*	230 ± 1.3*	219 ± 0.3*	216 ± 1.5*	211 ± 0.7*	201 ± 0.5*	186 ± 0.7*	180 ± 1.1*	176 ± 0.5*	168 ± 0.7*
G-VI	297 ± 0.7*	293 ± 0.6*	286 ± 0.5*	269 ± 0.5*	299 ± 0.6	291 ± 0.5	287 ± 0.7*	274 ± 0.5*	249 ± 1.5*	244 ± 1.0*	237 ± 0.8*	226 ± 0.9*	213 ± 1.2*	209 ± 1.1*	207 ± 0.7*	199 ± 0.5*	185 ± 1.9*	181 ± 0.7*	176 ± 1.1*	166 ± 0.7*

Two way ANOVA was applied and the values /data were presented in tabular form as a Mean ± standard error of mean. The results were summarized by comparing the experimental groups to disease groups and the normal group. The level of significance was shown in term of P-value ($P \leq 0.05$)

Effect of Extract on Body Weight of Diabetic Rats

To determine the effect of ethanolic extract of *Phyllanthus emblica* on body weight of diabetic rats, it was compared with standard drug glimepiride. There was a significant reduction in body weight in dexamethasone treated rats when compared to normal control. Treatment with the standard drug and ethanolic extract of *Phyllanthus emblica* in the dose 80 mg/Kg significantly prevented the loss in body weight. However *Phyllanthus emblica* in dose of 40 mg/Kg and 60 mg/Kg also prevented loss in body weight but the effect was dose dependent (Table IV).

Table IV: effect of ethanolic extract of *Phyllanthus emblica* on body weight of diabetic rats

Treatment Groups	Before Treatment Mean±SEM	After Treatment Mean ±SEM
Normal Control	215.66 ± 0.49	216.10 ± 0.71
Disease Control	248.76 ± 0.55	168.37 ± 0.83
Standard Control	274.50 ± 0.42	241.81 ± 0.94
***Phyllanthus emblica* 40mg/kg**	264.93 ± 0.66	190.33 ± 0.64
***Phyllanthus emblica* 60mg/kg**	271.69 ± 0.49	216.23 ± 0.91
***Phyllanthus emblica* 80mg/kg**	310.71 ± 0.57	274.11 ± 0.97

DISCUSSION

The current study showed the presence of medicinally active constituents in fruit extract of *Phyllanthus emblica*. The qualitative analysis revealed the presence of proteins and amino acids, carbohydrates, flavonoids, saponins, alkaloids, glycosides and steroids. Tannins and phenols were present but to a much lesser extent, whereas the quantitative analysis showed the percentage of the crude chemical constituents that exhibited the therapeutic and physiological activity.

Polyphenolic compounds found in a variety of medicinal plants are responsible for antidiabetic activity and cardio protective effects (Subramanian *et al.*, 1983). Moreover, it has been investigated that flavonoids are also responsible for hypoglycemic effects and they raise the levels of hemoglobin in rats (Sri *et al.*, 2013).

The fruit extract of *Phyllanthus emblica* possessed significant hypoglycemic activity and also it might have improved insulin resistance through enhanced insulin sensitivity in the peripheral tissues as was evident from decreased glucose levels.The tannins present in plant extractsare potential inhibitors of aldose reductase (AR) and they further oppose the pathways induced by oxidative stress as it was seen reversal of changes with reference to lipid peroxidation and protein carbonyl content and activities of antioxidant enzymes. *Phyllanthus emblica* also averted the accumulation of lens proteins which are triggered by hyperglycemia (Suryanarayana *et al.*, 2007). Aldose reductase shows a role in the development and progress of secondary complication of diabetes that also includes cataract(Daisy *et al.*, 2005), thus *Phyllanthus emblica* is supposed to prevent the levels of aldose reductase and protect the body from harmful effects of aldose in diabetic rats.

Findings further suggested thatdue to potent antioxidant activity, the plant extract is highly efficacious in controlling diabetes. The ability of tannins to augment glucose uptake and prevent adipogenesis, makes it potent remedy in treating non-insulin dependent diabetes mellitus (Kim *et al.*, 2010). It has also been stated that insulin obtained with chromium is proficient enough to reverse blood sugar, serum cholesterol and phospholipids levels to those of normal rats (Arts *et al.*, 2000).

CONCLUSION

It is concluded that ethanolic fruit extract of *Phyllanthus emblica* not only improves diabetes mellitus by increasing insulin sensitivity towards peripheral tissues but also reduces hepatic glucose outputs.

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