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EVALUATION OF ANTI-DIABETIC ACTIVITY OF COMMERCIALLY AVAILABLE EXTRACTS OF *PHYLLANTHUS EMBLICA* IN STREPTOZOCIN INDUCED DIABETIC RATS

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ABSTRACT

Phyllanthus emblica (syn. Emblica officinalis) or amla, is supposed to rejuvenate all the organ systems of the body, provide strength and wellness. It keeps us away from all the diseases by boosting our immune system. Besides, *Phyllanthus emblica* plant is reported to possess many pharmacological and clinical properties.Objective: To evaluate the anti- diabetic activity of commercially available extracts *Phyllanthus emblica* on high fat diet and low dose streptozocin induced diabetic rats.Materials and Methods: Diabetes was induced in albino rats using streptozocin 35 mg/kg i.p after giving high fat diet for 2 weeks. Animals with fasting blood glucose (FBG) above 200 mg/dl one week after injecting streptozocin were randomly divided into four groups of six rats each. Group 1- Diabetic rats, received only the distilled water (control),Group 2 - Diabetic rats, received *Phyllanthus emblica* crude extract (200 mg/kg/day)– *Phyllanthus emblica* Low dose (PEL), Group 3– Diabetic rats, received *Phyllanthus emblica* crude extract (400 mg/kg/day) – *Phyllanthus emblica* High dose (PEH), Group 4– Diabetic rats, received Glibenclamide (0.6 mg/kg/day) - Standard (S). All the animals received the respective drugs for six weeks. FBG was measured every 2 weeks till the end of sixth weeks by glucose-oxidase method.Results: Commercially available extracts *Phyllanthus emblica* showed significant fall in fasting blood glucose ($P<0.001$) from second week onwards in comparison to diabetic control group.Conclusion: The results suggest that commercially available extracts *Phyllanthus emblica* have significant hypoglycemic activity in diabetic model.

KEY WORDS: Diabetes Mellitus, Emblica Officinalis, Fasting Blood Glucose, Madhumeha, Indian Gooseberry, *Maharoga*

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INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiologies. It is characterized by chronic hyperglycemia together with disturbances of carbohydrate, fat and protein metabolism resulting from defects of insulin secretion, insulin action or both .[1]Ayurvedic System of medicine clearly defines 'Diabetes'. Diabetes mellitus was known to Indian Civilization since vedic period by the name *Asrava* (Prameha). Diabetes is also known as 'Madhumeha'. Diabetes is also called *Maharoga* (Major Disease) as almost all parts of the body and every cell of human physiology are affected. Synthetic drugs like sulfonylurea, biguanides, acarbose and insulin are widely used in the treatment of diabetes. The occurrence of side effects from prolonged administration of conventional drugs and recently evidence of cases of "insulin resistance", have triggered the search for safe and effective alternatives.Nowadays, scientists and researchers are very much trying on research of natural plant products all over the World and a large number of studies have shown the immense potential of medicinal plants used traditionally. The evaluation of medicinal plants used traditionally in treating diabetes is of growing interest. Therefore, nowadays, focus is given to an intensive search for novel type of antioxidants from numerous plant materials for management of diabetes without any side effects. *Phyllanthus emblica (syn. Emblica officinalis* the Indian gooseberry, or amla from Sanskrit amalika, is a deciduous tree of the family Phyllanthaceae. It is known for its edible fruit of the same name. Indian gooseberry has undergone preliminary research, demonstrating in vitro antiviral and antimicrobial properties.[2] *Phyllanthus emblica* primarily contains tannins, malic acid, astragalin, ellagic acid, gallotannin, kaempferol, kaempferol-3-o-glucoside, phyllantidine, phyllantine, rutin, phyllemblin, phyllemblic, gallic acid.[3,4] Its fruit juice contains highest vitamin C (478.56 mg/100ml). The fruit when blended with other fruits, boosted their nutritional quality in terms of vitamin C content.[5] Due to very rich source of vitamin C, it is also used as a medicine to prevent aging (rejuvenation) due to its strong antioxidant properties.[6] Rasayana (a group of herbal preparations including P. emblica) of the Indian traditional health care system, identified for their interesting antioxidant properties.[7] Extracts of *Emblica officinalis f*ruits possess cytoprotective and immunomodulating properties against chromium induced oxidative damage.[8] Indian gooseberry has undergone preliminary research, demonstrating in vitro antiviral and antimicrobial properties.[9] Previous studies suggest that this plant possess medicinal properties, but only a few of them have been done to establish their anti-diabetic properties.

MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology, Moti Lal Nehru Medical College, Allahabad. The study was carried out in albino rats of either sex weighing between 100 - 120 g[18]. Animals were obtained from registered animal seller (B-37/0605003769) and were kept in animal house of Moti Lal Nehru Medical College under the supervision of veterinary doctor. The animals were housed at an ambient temperature of 25^{o}C± 2°C with a 12- hr light/dark cycle, and provided with standard pellet diet/high fat and water ad libitum.The maintenance of the animals and the experimental procedures were in accordance with the guiding principles of Institutional Animal Ethics Committee and the 'Guide for the Care and Use of Laboratory Animals', National Research Council, 1996 (Latest revision in 2011). All the experimental procedures and protocols were reviewed and approved by Institutional Animal Ethical Committee (Project No. 63/IEC/ MLNMC/2013).

Test drugs and chemicals

All the drugs were given orally with the help of feeding cannula after suspension in distilled water (vehicle).

Streptozocin

(minimum assay 97%) was procured from Spectrochem Pvt. Ltd., Mumbai.Glucose estimation kit used for estimation of plasma glucose was purchased from Span Diagnostic Limited, Surat, India. All the chemicals and reagents used were of analytical grade.

Phyllanthus emblica extract

It was given in a dose of 200 mg/kg and 400 mg/kg[10]. It was procured from as commercially available crude extract in dry powder form, from The Himalaya Drug Co., Bengaluru India.

Glibenclamide

It was given in a dose of 0.6 mg/kg.[11] It was procured from USV Pharma Ltd, India.

Experimental Protocol

A total number of 24 rats were included in the study. Fasting plasma glucose (FPG) levels of all the rats were determined. All the animals were fed on high fat diet (58% energy as fat) for 2 weeks. After 2 weeks fasting plasma glucose levels were taken and all the rats taking high fat diet were injected with 35 mg/kg of streptozocin in citrate buffer, intraperitoneally (single dose).[12] The FPG levels were estimated in all the rats after 1 week high fat diet. The rats with plasma glucose level > 200 mg % were considered to be diabetic and were included in the study.[13] They were randomly (by using random number table) divided into 4 groups of 6 rats each; so that a total of 4 groups were formed as follows –

Group 1 - Diabetic rats, received only the distilled water (vehicle)-Diabetic Control (DC)
Group 2 - Diabetic rats, received *Phyllanthus emblica* crude extract in a dose of 200 mg/kg/day
Group 3 –Diabetic rats, received *Phyllanthus emblica* crude extract in a dose of 400 mg/kg/day
Group 4 –Diabetic rats, received Glibenclamide in a dose of 0.6 mg/kg/day.- Standard (S)

The test drugs were administered orally once daily after preparing suspension in distilled water for further 6 weeks. Fasting plasma glucose of all the rats were taken every 2 weeks. Blood samples were drawn from the tail vein and plasma glucose estimation was done by the Glucose-Oxidase method.

Statistical analysis

The observations were analyzed using ANOVA followed by dunnett's test to compare with control.

RESULTS

All the groups were observed during the study period as per the study requirements. Their basal fasting plasma glucose (FPG) levels were measured at the start of study. After feeding rats with their high fat diet for 2 weeks, FPG levels were again measured and they were injected with streptozocin in citrate buffer or plain citrate buffer depending upon the group as described in the methods section. One week after the injections, FPG levels were again measured and the diabetic status was ascertained. This reading was considered as that of zero weeks. Rats were continued on their high fat diet and drugs. FPG levels were determined every 2 weeks till the end of sixth week. The values of the test groups were compared with that of the control . The descriptive statistical values are depicted in Table 1-4.

Descriptive Statistics

Table 1
Results for group 1 (diabetic control)

Variable value	week	Mean	SE Mean	StDev
	week 0	359.50	8.23	20.16
	week 2	364.50	5.98	14.64
	week 4	370.50	5.23	12.80
	week 6	366.83	4.97	12.17

Table 2
Results for group 2 (phyllanthus emblica 200 mg/kg)

Variable value	week	Mean	SE Mean	StDev
	week 0	355.50	9.00	22.05
	week 2	339.17	8.55	20.94
	week 4	290.5	20.4	50.0
	week 6	288.33	7.13	17.47

Table 3
Results for group 3 (phyllanthus emblica 400 mg/kg)

Variable value	week	Mean	SE Mean	StDev
	week 0	358.17	3.94	9.64
	week 2	343.50	3.70	9.07
	week 4	301.50	2.22	5.43
	week 6	275.17	1.89	4.62

Table 4
Results for group 4 (glibenclamide)

Variable value	week	Mean	SE Mean	StDev
	week 0	356.33	6.88	16.86
	week 2	235.67	3.45	8.45
	week 4	182.17	3.32	8.13
	week 6	165.50	2.03	4.97

Table 5
One-way ANOVA: Value versus Group

Group	N	Mean	StDev	95% CI
1	24	365.33	14.81	(346.05, 384.62)
2	24	318.38	41.48	(299.09, 337.66)
3	24	319.58	34.45	(300.30, 338.87)
4	24	234.9	77.0	(215.6, 254.2)

(Pooled StDev = 47.5718)**On applying One way ANOVA for group wise comparison of means showed significant difference in means (P value= 0.0001). Standard deviation of the groups are depicted in Table 5 with a confidence interval of 95%. Pooled standard deviation was found to be 47.5718.**

Table 6
Dunnett Multiple Comparisons with a Control
(Grouping Information Using the Dunnett Method and 95% Confidence)

Group	N	Mean	Grouping
1 (control)	24	365.33	A
3	24	319.58	
2	24	318.38	
4	24	234.9	

(Means not labeled with the letter A are significantly different from the control level mean.)

Table 7
(Dunnett Simultaneous Tests for Level Mean - Control Mean)

Difference of Levels	Difference of Means	SE of Difference	95%	T-Value	Adjusted P-Value
2 - 1	-47.0	13.7	(-79.8, -14.2)	-3.42	0.003
3 - 1	-45.8	13.7	(-78.6, -12.9)	-3.33	0.004
4 - 1	-130.4	13.7	(-163.2, -97.6)	-9.50	0.000

Individual confidence level = 98.10%

The Dunnett test was applied for the comparison of the individual groups with the control(Group 1). On comparison of the Group 2, Group 3 and Group 4 with the control (Group 1) showed significant decrease in blood glucose level in all the groups (Group 2 vs Group 1-p=0.003, Group 3 vs Group 1-p=0.004, Group 4 vs Group1p=0.0001)with confidence interval of 95% confidence interval and at α=0.05(Table 7). The significant test is depicted pictorially in Figure 1.

Figure 1

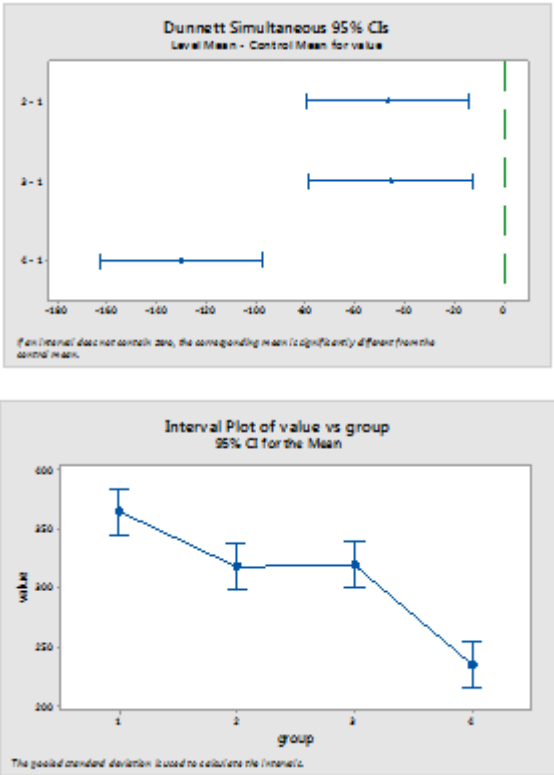


Table 8
One-way ANOVA: Value versus Week

week	N	Mean	StDev	95% CI
week 0	24	357.38	16.69	(333.49, 381.26)
week 2	24	320.7	52.8	(296.8, 344.6)
week 4	24	286.2	73.1	(262.3, 310.1)
week 6	24	274.0	74.0	(250.1, 297.8)

Pooled StDev = 58.9258 **On applying One way ANOVA for week-wise comparison of means showed there was significant lowering in blood glucose level when compared with value of week 0 (P value= 0.0001) and standard deviation of the groups are depicted in Table 8 with a confidence interval of 95%. Pooled standard deviation was found to be 58.9258.**

Table 9
Dunnett Multiple Comparisons with a Control
(Grouping Information Using the Dunnett Method and 95% Confidence)

week	N	Mean	Grouping
week 0 (control)	24	357.38	A
week 2	24	320.7	A
week 4	24	286.2	
week 6	24	274.0	

(Means not labeled with the letter A are significantly different from the control level mean.)

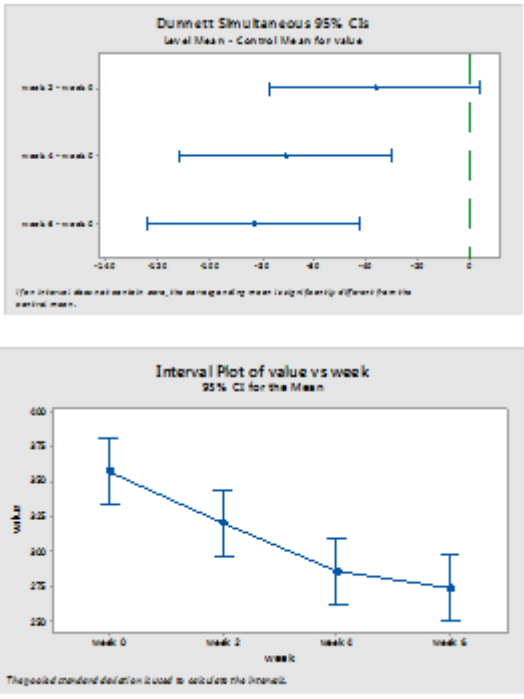
Table 10
(Dunnett Simultaneous Tests for Level Mean - Control Mean)

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
week 2 - week 0	-36.7	17.0	(-77.3, 4.0)	-2.16	0.086
week 4 - week 0	-71.2	17.0	(-111.8, -30.6)	-4.19	0.000
week 6 - week 0	-83.4	17.0	(-124.0, -42.8)	-4.90	0.000

Individual confidence level = 98.10%

The Dunnett test was applied for the comparison of the value at different weeks with the control (Week 0). On comparison of the values at Week 2 with the control (Week 0) did not show significant decrease in blood glucose level (p=0.08)but on comparison of values at Week 4 and Week 6 showed significant decrease in blood glucose level (p=0.0001) with a confidence interval of 95% and at α=0.05(Table 10). The significant test is depicted pictorially in Figure 2.

Figure 2



Effect of *Phyllanthus emblica* extract 200 mg/kg and 400mg /kg showed decreased blood glucose levels in experintal rats significantly from 4th week onward. The maximum net reduction in blood glucose level was seen at end of 6th week.The difference of blood glucose lowering effect of low dose (200mg/kg) and high dose (400mg/kg) *phyllanthus emblica* extract were not significant at all time points (p=1.00).Blood glucose lowering effect of *glibenclamide* was more significant when compared with low dose as well as high dose *phyllanthus emblica* extract(p=0.0001) at all time point.

DISCUSSION

The present study shows that antidiabetic effect of *Emblica officinalis* extract is significant when compare with diabetic control group. The antidiabetic effect is more sgnificant when we use extract in higher dose (400mg/kg).The improvement in plasma glucose level was consistent from second week onwards in both low as well as higher doses form of extract.Many medicinal plants are used by the population of developing countries as alternative therapy. In India, hundreds of plants are used traditionally for the management of diabetes mellitus. Unfortunately only a few of such medicinal plants have received scientific scrutiny.The

present study is supported by few other studies which is done earlier.Study done by Nain P etal. Shows that oral administration of the hydro-methanolic (20:80) extract of leaves of Emblica officinalis (HMELEO) at a concentration of 100, 200, 300 and 400 mg/kg b.w. daily for 45 days showed a significant (P<0.05) decrease in fasting blood glucose and increase insulin level as compared with the diabetic rats[14,] From the obtained results,it has been confirmed that aqueous fruit extract of Phyllanthus emblica and Curcuma longa has a potent antidiabatic activity by showing a significant fall(P<0.001) in blood glucose level of diabaic mice treated with aqueous fruit extract at 150 mg/kg body weight.Maximum decreased in blood glucose level was observed after 21 days of treatment.[15] Based on previous studies, various other mechanisms of Phyllanthus emblica extract may account for its anti-diabetic activity in our study. *Phyllanthus emblica* also shown to alter the key enzyme of glucose metabolism which may be responsible for its anti-diabetic effects. The antidiabetic effect of *Phyllanthus emblica* extract might be extra pancreatic either by inhibiting glycogenolysis, hepatic gluconeogenesis and glucose absorption from intestine or by increasing glucose absorption in cells of peripheral tissues (muscles and adipose tissues) and hepatic glycogenesis.[16] This finding supports the earlier reports of few Phyllanthus species, which were found to involve in regeneration and rejuvenation of β-cells leading to an increase insulin production and secretion.[15] *Emblica officinalis* and an enriched fraction of its tannoids are effectives in delaying development of diabetic cataract in rats. Aldose reductase has its involvement in the development of secondary complications of diabetes including cataract because Emblica officinalis is proved as an important inhibitor of aldose reductase.[17] In our study we have seen that compound possess antidiabetic activity, but the mechanism of their antidiabetic activity is not yet well elucidated. Therefore more work is needed in this direction. Another important finding of our study was the persistent improvement of plasma glucose levels in all the groups over 6 weeks. As this improvement continued till 6 weeks, studies designed to monitor glucose over longer periods can be done.

CONCLUSION

The following conclusions are drawn after the completion of study –

- ➢ *Phyllanthus emblica* was found to possess antidiabetic effect and antidiabetic effect is more significant with higher dose of extract.
- ➢ The improvement in plasma glucose levels was consistent from 4th week onwards in both the dose groups and our standard drug Glibenclamide showed better glucose lowering effects than *Phyllanthus emblica* at all the doses which we were used.

CONFLICT OF INTEREST

Conflict of interest declared none.

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