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HAEMOLYTIC ACTIVITIES AND ANTIDIABETIC EFFECT OF TERMINALIA ARJUNA AND *EMBLICA OFFICINALIS*

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ABSTRACT

Objective- To investigate anti-diabetic and haemolytic activity of aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis*. **Methods-** Antidiabetic bioassay was done through estimation of blood counts, total cellular (i.e. proteins) and free haemoglobin content in diabetic blood plasma and also determined its haemolytic activity in human whole blood. **Results-** Overall, the results suggest that aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* showed anti-diabetic activity with respect to enhancement of granulocytes count and decrease in free haemoglobin content including total cellular content in diabetic human whole blood and plasma samples. **Conclusions-** *Terminalia arjuna* and *Emblica officinalis*, aqueous stem bark extract revealed the presence of bio-active constituents which are known to exhibit anti-diabetic activities.

KEYWORDS: *Terminalia arjuna; Emblica officinalis*; aqueous stem bark extract; haemolytic; diabetic.

INTRODUCTION

According to traditional system of medicine, medicinal plant products have been used continuously for treating various infections including cardiovascular diseases especially diabetes.[1, 2] Due to its availability of anti-diabetic drug (type 1, huminsulin or 2, oral drugs) that are accessible but still continued to be a major medical health problem.[3, 4] Recently, some medicinal plant products have been reported (preclinical or phase 1 trial) as anti-diabetic agent worldwide.[5] As per the literature, more than 400 medicinal plant species having anti-diabetic activity.[5]

However, searching for new antidiabetic drugs from medicinal plants is still attractive because they contain substances (primary and secondary metabolites) which demonstrate alternative and safe effects on diabetes mellitus. Most of medicinal plants[6] contain primary (e.g. protein) and secondary metabolites (i.e. alkaloids, terpenoids, flavonoids etc.) that are regularly implicated as having antidiabetic effect. Most effective and commonly studied medicinal plants in relation to diabetes (including its complications) are *Ficus benghalensis*, *Calotropis gigantea*, *Syzygium cumini* etc. [5, 7, 8, 9]

Over the last three decades, immunological or cardiovascular chronic disorders i.e. diabetes emerged as the major causes of adult morbidity and mortality rate in India. Among these we have evaluated aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis*; both these medicinal plants have shown varying degree of anti-diabetic activity.

Terminalia arjuna (evergreen tree; family *Combretaceae)*, medicinal plant commonly found in Deccan regions, sub-Himalayan tracts and grows predominantly besides water channels. Traditionally, this medicinal plant is used for treating various heart diseases. In addition, this medicinal plant also showed some properties i.e. anti-inflammatory, anti-cancer, blood thinning etc.[10, 11, 12]

Emblica officinalis (family *Euphorbiaceae*), medicinal plant and commonly used in cough, asthma, bronchitis, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, inflammations, anaemia, emaciation, jaundice, diarrhoea, dysentery etc. In contrast, this medicinal plant also showed various properties i.e. anti-inflammatory, anti-oxidant, adjuvant etc.[13, 14, 15] The current study was focused only on anti-diabetic and haemolytic activity of aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* on human diabetic blood samples.

MATERIALS AND METHODS

Plant material and its preparation

Stem bark of *Terminalia arjuna* and *Emblica officinalis* were collected from the garden (reference http://www.vsbt.res.in/nakashtra.php) of Vidya Pratishthan's School of Biotechnology, Baramati,

District Pune, India. In this study, fresh stem bark of these two medicinal plants were dried at room temperature and then prepared in a powdered form. Powdered stem bark were macerated (using mortar and pestle) and dissolved in phosphate buffered saline (pH, 7.2). Finally, the samples were centrifuged at high speed (10000 rpm) and collect the supernatant for various immunopharmacological based studies.

Preliminary screening of secondary metabolites

The aqueous extracts were utilized for primary screening (qualitative) of different secondary metabolites i.e. alkaloids, flavonoids, terpenoids, tannins, sterols etc. by using standard protocols.[16]

Identification of these secondary metabolites in the aqueous stem bark extract of *Terminalia arjuna and Emblica officinalis* revealed the presence of alkaloids, tannins, carbohydrates, saponins, terpenoids and flavonoids.

Estimation of blood counts

To determine the immunopharmacological (anti-diabetic) studies of aqueous stem bark extract of *Terminalia arjuna and Emblica officinalis* in human diabetic whole blood samples. These blood samples (anti-coagulant EDTA, diabetic) were collected from *Mangal Pathology Laboratory*, Maharashtra, India and analysed or processed at the VSBT, Baramati, Maharashtra, India, between October to December 2015. In this study, variable doses of aqueous stem bark extract (0.5 – 30 mg/ml, 100 µl) of *Terminalia arjuna* and *Emblica officinalis* in human whole blood (100 µl) for 2 h incubation at carbon dioxide incubator (37 °C and 5 % CO_2) in order to estimate the total blood counts (lymphocytes, monocytes and granulocytes count) using forward and side scatter.[8]

Huminsulin 50/50 used as standard for these studies. After incubation, lysed the diabetic human blood samples (using red cell lysis buffer) and washed with phosphate buffered saline (PBS; pH 7.2). Finally, these samples were examined as well as scrutinized through flow cytometer using forward and side scatter (FACS Calibur, BD Biosciences).

Total cellular and free haemoglobin content

In this study, 100 µl of diabetic human whole blood (free haemoglobin; glycosylated) or plasma (total cellular content) samples were taken in each tube (two different set of experiments). Add serial dilutions of aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* (0.5 – 30 mg/ml, 100 µl) on human whole blood and plasma. Incubated the samples in dark for 2 h at 37°C carbon dioxide incubator. Subsequently, 2 ml of red cell lysis was added and incubate at room temperature for 10 min. The samples were spinned (2500 rpm, 4ºC) and the supernatant was aspirated/eliminated and washed with PBS.[8] After centrifuging, pellet containing aqueous stem bark extract dissolved in PBS and observed the cells through UV visible spectrophotometer.

Haemolytic activity

For these studies, 1% red-blood cell suspension of diabetic human whole blood in pH 7.2, PBS was used throughout the experiment. Aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* were prepared and dissolved in PBS buffer. These samples of different concentrations (0.5-30 mg/ml; 100 µl) containing a fixed volume of red-blood cell suspension. Incubate the samples for 3-4 h at carbon dioxide incubator. Lysis and washing the diabetic blood samples containing variable concentration of aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis*. Haemolytic activity of aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* was evaluated. Distilled water used as negative control in red-blood cell suspension. The optical density (OD) was measured at 570 nm.[17]

RESULTS

Blood counts

In order to estimate the total blood counts (lymphocytes, monocytes and granulocytes) in diabetic human whole blood samples using variable doses of aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* using flow cytometry as shown in **Table 1**. The flow cytometric results showed that aqueous stem bark extract at a dose range of 0.5 mg/ml showed some fluctuations in the number of total blood counts (increased in the number of granulocytes and lymphocytes but decrease in monocytes count) as compared to control. Huminsulin 50/50 used as standard for these studies and the results showed that there is also increased in blood counts (lymphocytes and granulocytes count) as compared to control. Overall, aqueous stem bark extract showed anti-diabetic activity.

Total cellular and free haemoglobin content including haemolytic activity

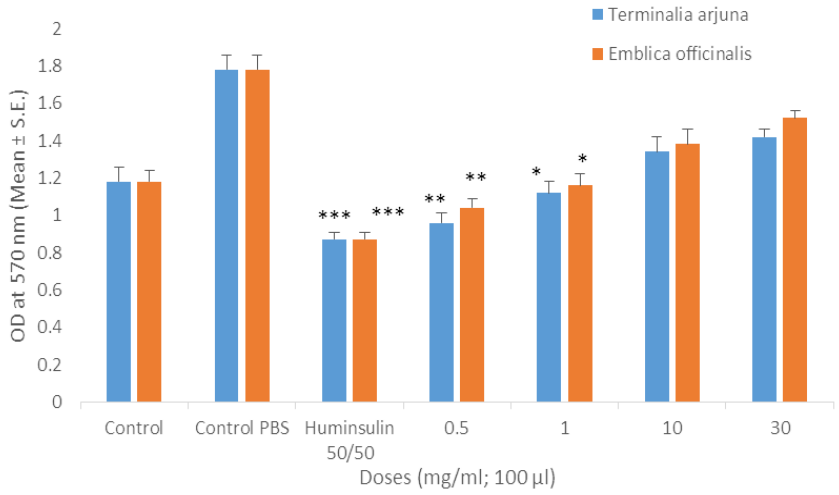
The effect of aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* on lysed human diabetic blood and plasma samples for the estimation of free haemoglobin and total cellular content as shown in **Fig. 1**. The results showed that aqueous extract (at dose range i.e. 0.5 mg/ml) and huminsulin 50/50 decreased free haemoglobin and total cellular content as compared to control. In addition, aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* showed less haemolytic activity (**Fig. 2**.)as compared to distilled water (used as negative control).

Table 1. Effect of variable doses of aqueous stem bark on diabetic human blood counts using flow cytometry

Plant material	Doses (mg/ml; 100 µl)	Lymphocytes	Monocytes	Granulocytes
Terminalia arjuna	Control	3.78 ± 0.54	2.54 ± 0.06	16.46 ± 2.88
	Control PBS	7.98 ± 1.08	6.34 ± 0.76	21.26 ± 1.54
	Huminsulin 50/50; 10 µl	13.64 ± 2.56**	2.34 ± 0.44**	34.8 ± 3.48**
	0.5	11.4 ± 1.14*	3.12 ± 0.52*	42.8 ± 4.12***
	1	10.8 ± 1.02	4.78 ± 0.88	41.2 ± 2.88
	10	6.4 ± 0.54	4.82 ± 0.54	30.3 ± 2.84
	30	5.14 ± 0.78	4.96 ± 0.68	20.6 ± 1.88
Emblica officinalis	0.5	8.78 ± 1.78	3.98 ± 0.52	38.4 ± 4.2**
	1	7.04 ± 1.46	5.12 ± 0.98	35.8 ± 2.8
	10	6.88 ± 0.86	4.82 ± 0.54	26.4 ± 1.98
	30	5.82 ± 0.66	3.44 ± 0.42	22.6 ± 1.4

Flow cytometric analysis of aqueous stem bark (0.5 – 30 mg/ml; 100 µl) extracted from *Terminalia arjuna* and *Emblica officinalis* for determine its effect in diabetic human whole blood samples containing lymphocytes, monocytes and granulocytes count. Data acquisition of 10000 events and fraction or separation of cell populations representing different phenotypes analysed using cell quest software. *P<0.05; **P<0.01 and ***P<0.001

a) Free Haemoglobin content



b) Total cellular content

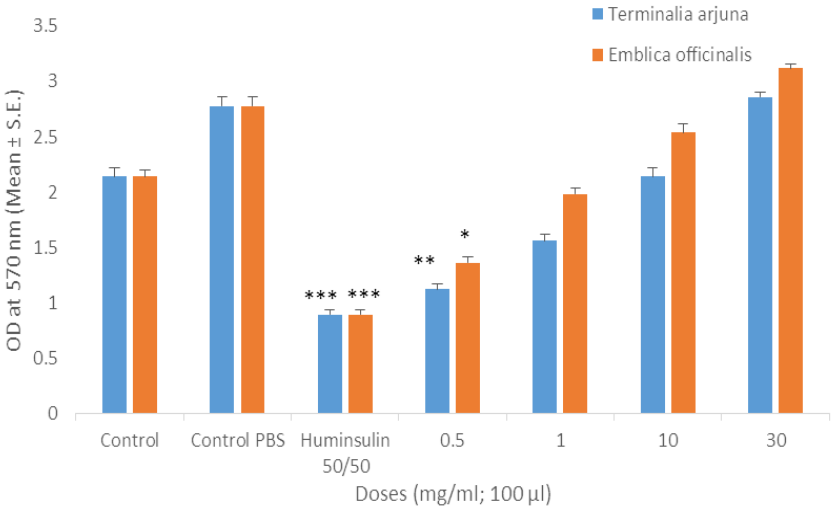


Fig.1. Estimation of free haemoglobin (glycosylated) and total cellular content in human plasma samples. Values are expressed as Mean ± S.E. *P<0.05; **P<0.01 and ***P<0.001

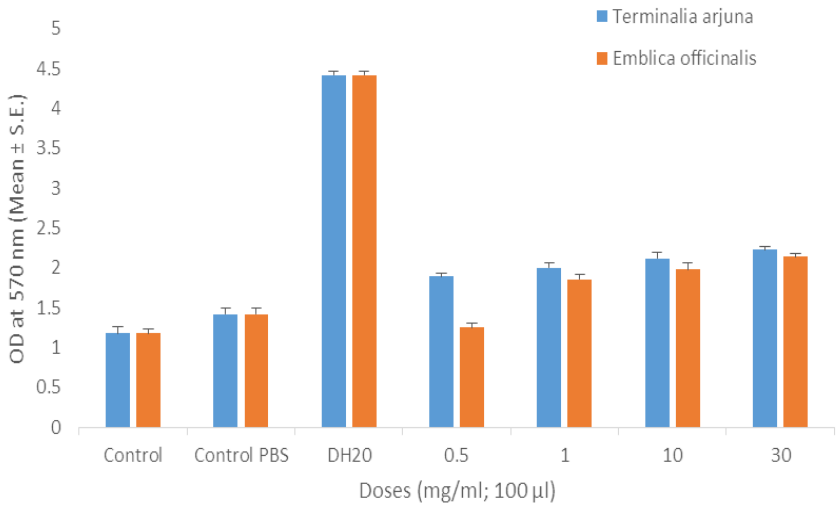


Fig.2. Haemolytic activity of aqueous stem bark on human erythrocytes. Data are represented as Mean ± S.E. of five human whole blood samples. Distilled water and phosphate buffered saline used as positive and negative control.

DISCUSSION

One of the chronic human diseases i.e. Diabetes in which the body cannot be able to regulate the amount of sugar in the blood and is generally caused by too little insulin (Type 1) or resistance to insulin (Type 2) or both. Recently, scientists focused on various medicinal plants which have the potential to prevent the burden of cardiovascular disease i.e. diabetes e.g. glucophage (metformin; hypoglycemic drug) derived from *Galega officinalis*.[18] A wide array of medicinal plant products containing phytochemicals i.e. alkaloids, glycosides, polysaccharides, flavonoids, steroids and terpenoids have demonstrated bioactivity against hyperglycemia.[19, 20]

Ultimately, these medicinal plants have the potential to prevent the burden of Type 1 and 2 diabetes. Recently, medicinal plants have been the primary source of drugs and most of the currently available drugs have been directly or indirectly derived from plants. In the present study, we validate the antidiabetic properties of aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* and identify the phytochemicals that are present in the extract.

In human diabetic blood samples, granulocytes played a significant role in the host inflammatory response against different types of infection (intracellular or extracellular). Several immunopharmacological studies have suggested or claimed that inhibition in the count of granulocytes in human whole blood which contributes the acceleration rate of diabetes and showed its devastating effect on various body organs.[3, 4, 8]

In this study, aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* showed enhancement in granulocytes count as compared to diabetic control samples. Lot of research work is already done as well as reported in animal model based studies especially rats and mice which showed declining rate of phagocytosis, neutrophil migration and hydrogen peroxide production.[8] Furthermore, the reduction of blood glucose levels through insulin (i.e. Huminsulin 30/70 or 50/50) treatment of diabetic patients has been reported to be significantly correlated with improvement of neutrophil phagocytosis capacity or granulocytes count.

Glycated haemoglobin means haemoglobin exposure to plasma glucose and is formed in a non-enzymatic glycation pathway. In diabetes, average amount of plasma glucose increases, the fraction of glycated haemoglobin (i.e. free haemoglobin in plasma) also increases in a predictable way. In this study, treatment of variable doses of aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis* on diabetic human plasma samples and determined total cellular content (i.e. proteins) and free haemoglobin in the supernatant through UV visible spectrophotometer.

The results showed that aqueous stem bark declined in the total cellular content and free haemoglobin in the supernatant at lower doses as compared to huminsulin 50/50. In addition, these aqueous extracts showed dose dependant increase in haemolytic activity Overall, the results of aqueous stem bark showed anti-diabetic activity in human whole blood and plasma samples.

CONCLUSION

In conclusion, these results provide evidence for the anti-diabetic activities of aqueous stem bark extract of *Terminalia arjuna* and *Emblica officinalis*. Further experimental and clinical trial studies are now warranted to investigate the benefit of aqueous stem bark extract treatment for diabetic patients.

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