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IN-VITRO ANTI-DIABETIC EVALUATION OF *TERMINALIA TOMENTOSA* (Roxb). BARK

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ABSTRACT

Diabetes mellitus is a disease caused by the imbalenced carbohydrate, fat and protein metabolism, caused due to either lack of insulin secretion or decreased sensitivity of the tissues to insulin, and is characterized by chronic raise in blood sugar levels termed as hyperglycaemia. The present work demonstrate the *in vitro* antidiabetic activity of Ethanolic and Aqueous extract of *Terminalia tomentosa* stem bark. The extracts were studied for their effect on inhibition of glycosylation of haemoglobin, and α-Amylase inhibition. Inhibition of glycosylation of haemoglobin and α- Amylase inhibition was found to be in a dose dependent manner. The results obtain confirms *Terminalia tomentosa* bark possesses antidiabetic activity. However, further studies on the extract and isolated constituents to be carried out on *in vivo* models and clinical trials for its effective utilization as therapeutic agents.

Keywords: *Terminalia tomentosa*, antidiabetic, Acarbose,glycosylation.

INTRODUCTION

Many research and investigations of oral anti-hyperglycaemic agents of natural plant origin were used in traditional medicine have been studied and many of them have been found to posess the positive activity[1,2]. Diabetes mellitus is a syndrome of imbalenced carbohydrate, fat and protein metabolism, caused due to either lack of insulin secretion or decreased sensitivity of the tissues to insulin,and is characterized by chronic hyperglycaemia[3,2]. The worldwide incidence of diabetes has risen in the past two decades. Type 2 diabetes is more

common, and its prevalence is expected to rise more rapidly in the future because of increasing obesity and reduced activity levels. Inspite of several new pharmacologically active agents have been developed for the management of diabetes, the treatment of diabetes with herbal remedies has also been increasing among practitioners. Ancient Indian literature has prescribed various herbs in the treatment of diabetes mellitus. Many indigenous drugs have been used by the practitioners of the Ayurvedic system for the treatment of diabetes mellitus in India [4, 2].

Terminalia tomentosa, commonly known as "Asaina" in Hindi, "Black murdah" in English and "Sadad" in Gujrat, belongs to *Combretaceae* family[5], is a large tree found in deciduous forests. It can only be identified by its scissred and cracked bark and for this reason is sometimes known as crocodile bark tree.

The bark is reported to be used in diarrhea, dysentery, astringent, cough, bronchitis, verminosis, leucorrhoea, gonorrhea and burning sensation. Economically the bark is used for dyeing black and it yields a gum which is used as an incense and cosmetic. Ash yielded by the calcareous matter in the trunk of this tree, is used by chewers of betel nut[6].

As there is no scientific study has been reported as per available literature for its antidiabetic property, the present study has been carried out to evaluate the possible antidiabetic activity by in vitro models.

MATERIAL AND METHODS

The stem bark of *Terminalia tomentosa*.were collected from Someshwara forest ,Hebri, Manipal, Karnataka, India during August 2012 and were authenticated by Dr.Gopala Krishna Bhat, taxonomist , Poorna Prajna College Udupi.

Preparation of extracts

100 g of the shade dried bark powder was extracted with ethanol by hot extraction process for 72 h. After completion of the extraction the solvent was recovered by distillation and concentrated *in vacuo.* The aqueous extract was prepared by maceration process with 100 g of the stem bark powder using chloroform:water (1:99) for seven days, after completion of the extraction the solvent was recovered by distillation and concentrated *in vacuo*.

***In-vitro* Antidiabetic activity**

Non-enzymatic glycosylation of haemoglobin method [7]

Antidiabetic activity of bark of *Terminalia tomentosa* were investigated by estimating degree of non-enzymatic haemoglobin glycosylation. To 1 ml of haemoglobin solution 5µl of gentamycin and 25µl of Gallic acid (as standard) 25 µl of the bark extract of *Terminalia tomentosa* was added. The reaction was started by the addition of 1 ml of 2% glucose in 0.01M phosphate buffer pH 7.4 and incubated in the dark at room temperature. The concentrations of glycated haemoglobin at the incubation period of 0, 24 and 72 h, were estimated colorimetrically at 443 nm (Adisa*et al*., 2004). The test was conducted in triplicate and % of inhibition was calculated by

$$\% \text{ inhibition} = \frac{As - Ac}{As} * 100$$

Ac - Absorbance of Control

As- Absorbance of Sample

All determinations were carried out in triplicates

A- Amylase inhibition[8, 9]

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitory activity was based on the starch iodine method thatwas originally developed by Fuwa1954[10]and later employed by others for determination of amylase activity in plant extracts[11]with some modifications .In alpha amylase inhibition method 1ml substrate- potato starch (1%w/v), 1 ml of drug solution (Acarbose std drug/ ethanolic extract/ aqueous extract) of different concentration, 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) was added.NOTE- Potato starch solution, alpha amylase solution and drug solution was prepared in acetate buffer (820.3 mg Sodium acetate and 18.7mg sodium chloride in 100ml distilled water). The above mixture was incubated for 1 hr. Then 0.1 ml Iodine-iodide indicator (635mg Iodine and 1gm potassium iodide in 250ml distilled water) was added in the mixture. Absorbance was taken at 565 nm in ELISA.

$$\text{Inhibition of alpha- Amylase (\%)} = \frac{As - Ac}{As} * 100$$

Ac - Absorbance of Control

As- Absorbance of Sample

All the experiments were carried out in triplicates.

RESULTS AND DISCUSSION

*In-vitro*Non-enzymatic glycosylation of haemoglobin method

The aqueous and ethanolic extracts of *Terminalia tomentosa* bark exhibits potent antidiabetic activity.The percentage inhibition of glycosylation was found to be dose dependent.As the concentration of drug increases formation of glucose-haemoglobin complex decreases and free haemoglobin increases, which show the inhibition of glycosylated haemoglobin.In our experimental study it was observed that ethanolic and aqueous extract of*Terminalia tomentosa* bark demonstrated significant activity.

Table 1:*In-vitro*Non-enzymatic glycosylation of haemoglobin method

S.No	Concentration (µg/ml)	% GLYCOLISATION		
		STD	T.A	T.E
1	10	6.58	8.94	3.51
2	20	19.32	21.48	19.26
3	40	22.83	32.38	28.56
4	80	42.74	40.18	39.36
5	160	48.92	59.85	61.04
6	320	55.63	64.37	64.38

T.A- T.tomentosa aqueous extract, T.E- T.tomentosa ethanolic extract

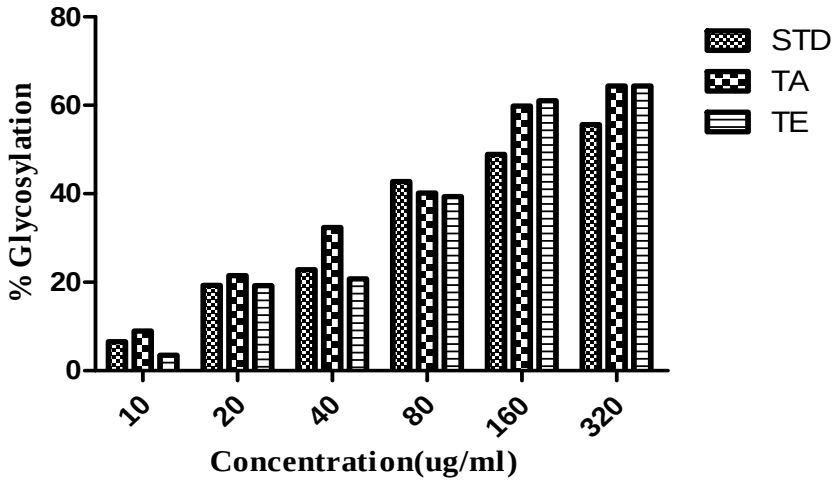

Fig. 1 Non-enzymatic glycosylation of Haemoglobin

Alpha- amylase inhibition method

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in to mono and disaccharide. In our experimental study it was observed that ethanolic and aqueous extract of *Terminalia tomentosa* bark demonstrated significant Alpha amylase inhibition activity as compared to standard drug acarbose.

Table 2: Alpha Amylase inhibition

S.No	Concentration (µg/ml)	% Inhibition		
		Std	T.A	T.E
1	10	20.46	19.56	23.43
2	20	36.65	32.89	33.08
3	40	44.01	48.83	38.09
4	80	55.41	54.89	57.2
5	160	65.81	66.58	61.78
6	320	78.45	74.65	72.01

T.A- T.tomentosa aqueous extract, T.E- T.tomentosa ethanolic extract

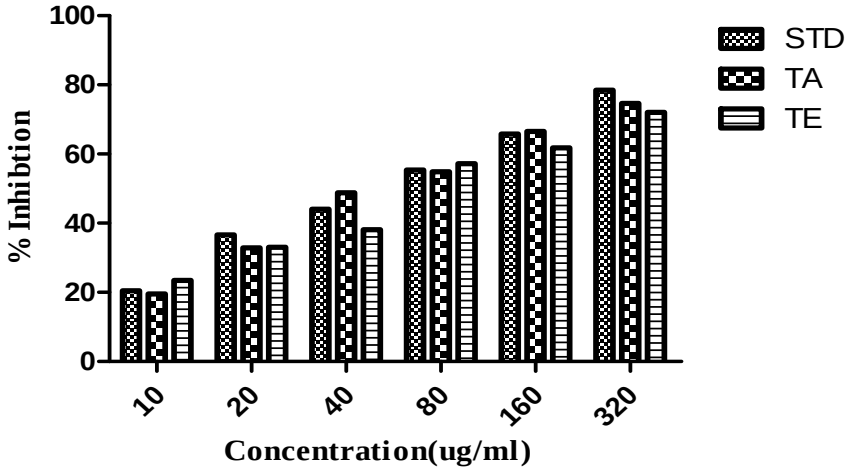

Fig. 2 Alpha amylase inhibition method

ABBREVIATIONS

STD**= Standard**

T.A= *Terminalia tomentosa* aqueous extract

T.E =*Terminalia tomentosa* ethanolic extract

Abs=Absorbance

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