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THE VITRO ANTI-DIABETIC ACTIVITY OF METHANOLIC AND ETHANOLIC EXTRACT OF ARGIMONA MAXICANA

Reshma Mane-Deshmukh[1]

[1]SPM'S College of Pharmacy (Degree) Malewadi, Akluj, Dist- Solapur, Maharashtra, India

Jyoti Ganjkar[2]

[2]SPM'S College of Pharmacy (Degree) Malewadi, Akluj, Dist- Solapur, Maharashtra, India

Shital Gaikwad[3]

[3]SPM'S College of Pharmacy (Degree) Malewadi, Akluj, Dist- Solapur, Maharashtra, India

Tejaswini Fugare[3]

[3]SPM'S College of Pharmacy (Degree) Malewadi, Akluj, Dist- Solapur, Maharashtra, India

ABSTRACT

Diabetes mellitus is a complex disease that changes the metabolism of carbohydrate, fat and protein. The alpha-amylase is intestinal digestive enzyme and plays a vital role in the carbohydrate digestion. There is antidiabetic therapeutic approach which reduces glucose level in blood by the inhibition of alpha-amylase enzyme. This is important strategy in management of blood glucose level. The aim of the present study was to investigate in-vitro antidiabetic activity of methanolic and ethanolic extract of Argimona maxicana. The antidiabetic activity was compared with standard drug Acarbose. Results obtained indicate that methanolic and ethanolic extract of Argimona maxicana possessed significant in-vitro antidiabetic activity. The extract exhibit the dose-dependent increase in inhibitory effect on alpha-amylase enzyme (upto 92.3 %).the methanolic extract possessed more significant activity than ethanolic extract of Argimona maxicana. However, these effects need to be confirmed by clinical trials for its effective utilization as therapeutic agents

KEYWORDS: *Argimona maxicana; In-vitro antidiabetic activity; alpha-amylase*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, which leads to various acute and chronic complications if it is untreated and may cause damage to the renal, cardiovascular, retinal and neurologic systems. It occurs due to relative or absolute lack of insulin, or its action on the target tissue, or both (Marles RJ; 1995 and Bracken P; 2003). The prevalence of diabetes is increasing annually and the number of diabetics is projected to rise above 300 million before 2025(Ganiyu O; 2012). One important factor which result in to postprandial hyperglycemia is the fast uptake of glucose in the intestine by the action of α-amylase that helps in the breakdown of complex carbohydrates into simple sugars such as maltose and glucose (Hua-Qiang D.; 2012 & Gray DM; 1995). Many synthetic antidiabetic agents are currently available but they are either too expensive or produce side effects on chronic use (Rang HP; 1991).

Traditionally, many indigenous plants have been used successfully for the management of the disease throughout world. *Argemone mexicana* (Linn.) belongs to Family *Papaveraceae* and is commonly known as Mexican poppy or Prickly Poppy. The whole plant, roots, leaves, stem, flowers are extensively used in traditional system of medicine for various ailments like leprosy, malaria, jaundice, rheumatism, pain, inflammation, skin diseases, fever, piles, warts, dysentery, tumors and worm infestations (Warrier PK;1996, Chopra RN;1979, Nadkarni KM;1976). The plant is known to possess antimalarial (Gehlot D;2000), antimicrobial (Gehlot D;2000), antibacterial (Indranil Bhattacharjee;2006) and antifungal (Sreejayan N;1996) activities. In Mexico infusion of aerial part of the plant is used as hypoglycemic (Adolfo Andrade-Cetto;2005). Chemical investigations of this plant have revealed the presence of alkaloids (Hussain SF;1983,& Nakkady S;1988) , amino acids (Dinda B;1986), phenolics (Harborne JB;1983) and fatty acids (Gunstone FD;1977).

The in-vivo study of *A. maxicana* showed significant antidiabetic acivity and The effect is more significant at a higher dose (S.P.ROUT;2011) Hence, the present study has been designed to determine the effectiveness of ethanolic and methanolic extracts of *A. mexicana* in in-vitro antidiabetic's activity by using alpha amylase enzyme.

MATERIAL AND METHODS

Collection of plant material:-

The whole plant parts of *Argimona maxicana* of family papaveraceae was collected from local area of akluj in month of august 2016 and were authenticated by botany Department, S.M.M. college akluj, Maharashtra.

Extraction of Plant material :-

The aerial parts were dried under shade condition and made into coarse powder using a mechanical grinder. The powdered material was defatted with petroleum ether for 72-hours and extraction with methanol and ethanol using cold maceration process for 72-hours. The extract was filtered and concentrated by rotary evaporator and kept in vacuum desiccators until use. The yield of the extract was 9.68% w/w with respect to dried powder.

Preparation of Extract and Standard:-

The measured quantity of extracts and fractions of *A. mexicana* and the standard drug Acarbose was suspended in 25% tween-20 in distilled water

Inhibition of alpha-amylase enzyme:-

A starch solution (0.1% w/v) was obtained by stirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution 96 mM. Both control and plant extracts were added with starch solution and left to react with alpha- amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3- amino-5- nitro salicylic acid. This reaction is detectable at 540 nm (Malik and Singh, 1980).

% inhibition = A_c-A_s/A_c X 100

Where Ac is the absorbance of the control and As is the absorbance of the sample.

Statistical Analysis:-

The results are expressed as mean ± SEM.data was analyzed by One-way ANOVA and compared by Dunnett's multiple comparison tests. Confidence Interval has been considered as 95% and $p < 0.01$ were considered significant

RESULT AND DISCUSSION

Table1: Absorbance of standard, MEAM and EEAM

Blank	Conc (µg/ml)	Standard	MEAM	EEAM
0.038± 0.02	100	0.0167± 0.05	0.0038**± 0.05	0.0120**±0.00
	200	0.0132± 0.05	0.0037**± 0.05	0.0110**± 0.05
	300	0.0101± 0.05	0.0035** ± 0.05	0.0098**± 0.05
	400	0.0089 ± 0.008	0.0033**± 0.003	0.0099**±0.01
	500	0.0072± 0.007	0.0029**± 0.003	0.0082**±0.008

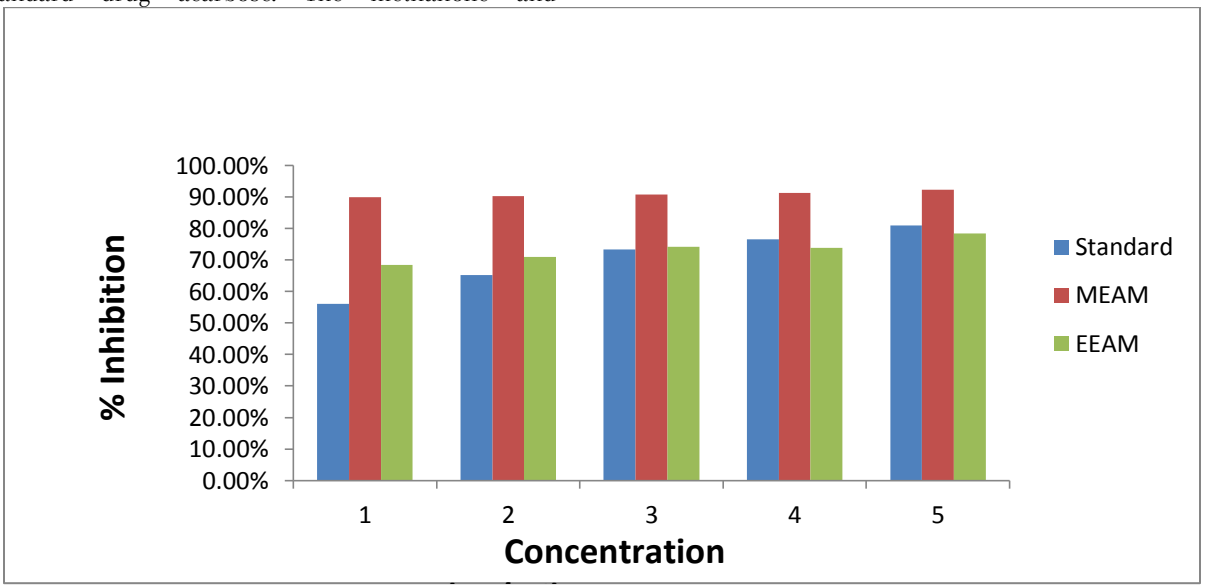
Values are expressed as Mean ± SEM; (n = 3); One Way ANOVA followed by dunnett Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.00 *vs.* standard Group MEAM-Methanolic Extract of Argimona Maxicana ; EEAM- Ethanolic Extract of Argimona Maxicana

Table2: % Inhibition of α amylase

Conc (µg/ml)	Standard	MEAM	EEAM
100	56.05%	90%	68.42%
200	65.26%	90.26%	71.05%
300	73.42%	90.78%	74.21%
400	76.57%	91.31%	73.94%
500	81.05%	92.36%	78.42%

MEAM-Methanolic Extract of Argimona Maxicana ; EEAM- Ethanolic Extract of Argimona Maxicana

The in-vitro antidiabetic acivity was studied by using α-amylase.the ethanolic and methanolic extract of *Argimona maxicana* showed significant antidiabetic activity (p <0.01) when compared with standard drug acarbose. The methanolic and ethanolic extract of concentration 100 µg/ml showed 90% and 68.42% inhibition of α-amylase whereas 500 µg/ml showed 92.36% and 78.42% inhibition of α-amylase. But the methanolic extract of *Argimona maxicana* showed more % inhibition of α-amylase than ethanolic extract of *Argimona maxicana*



Graph1: % inhibition of standard, MEAM and EEAM vs concentration (1=100 µg/ml 2=200 µg/ml, 3=300 µg/ml, 4=400 µg/ml, 5=500 µg/ml)

CONCLUSION

From present study, we found that Methanolic and ethanolic extract of *Argimona maxicana* shows significant in-vitro antidiabetic activity. But methanolic extract of *Argimona maxicana* shows more % inhibition of α-amylase than ethanolic extract of *Argimona maxicana*

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