PHYTOCHEMICAL SCREENING AND ANTI-DIABETIC EFFICACY OF STEM OF MAYTENUS EMARGINATA (WILD)

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ABSTRACT : In this present study we were focused on the possible anti-diabetic efficiency of the plant "Maytenus emarginata" in laboratory animals and the statistical significance of such effect.The plant extract was subjected to evaluation of gastrointestinal motility and anti-diabetic efficacy through determining Disaccharidase activity and six segment method which was performed to determine the amount of sucrose remaining in the GIT at six different positions. In the GI motility test, the extract exhibited laxative effect, which results the diminished absorption in small intestine. In Six Segment test, the quantity of sucrose unabsorbed in different GIT segments were estimated in control rats vs. rats administered with 500mg/kg extract at 30 minutes, 60 minutes, and 120 minutes. In Dissacharide activity the quantity of unabsorbed sucrose in Pancreatic Enzymes are determined in control rats vs rats administered with 500mg/kg extract. The extract caused a significant, dose reliant inhibition of glucose absorption and produced hypoglycemic activity in Long-Evans rats weighing about 100-200 gm. The anti-diabetic efficacy was assayed by measuring the amount of glucose in the samples collected after the experiment.In this study, the observations provide conformation and possible mechanisms of action for the anti-diabetic properties of plant "Maytenus emarginata" claimed in Ayurveda medicine.

Keywords: Anti-Diabetic, Maytenus emarginata, hypoglycemic, Glucose, Sucrose, Disaccharidase.

I.INTRODUCTION

Diabetes mellitus (DM) is a serious health problem being the third greatest cause of death all over the world, and if not treated, it is responsible for many complications affecting various organs in the body[1] (El-Hilaly et al., 2007). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs[2] (Lyra et al., 2006). In diabetic rats, the utilization of impaired carbohydrate leads to accelerate lipolysis, resulted in hyperlipidemia[3] (Morel and Chisolm, 1989). Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies[4] (Mitra et al., 1996). More than 400 plant species having hypoglycemic activity have been available in literature[5,6] (Oliver-Bever, 1986; Roy et al., 2005), however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on DM.

*M. emerginata*is belongs to family Celastraceae distributed throughout the arid, dry areas of India like Punjab, Sind, and Gujarat and also in Afghanistan, Arabia, Mediterranean, Tropical Africa, Malaya and Australia. In differnt Ayurvedic literatures such as Nighantu Adarsh,[7]Vanaspati Shashtra,[8,9]Aryabhishek,[9]Vasundhrani vanaspatio[10]various uses of the plant M. emerginatahas been mentioned. It is believe to be use for treatment of jaundice,[11,7]inflammation and rheumatic pain,[11,7,9]corneal opacity,[7-9]ulcers, gastrointestinal disorders, dysentery, toothache and also as a vermifuge.[11,12,13,14]

II.MATERIALS AND METHODS

Reagents and Apparatus

Surgical apparatus, Ice cold saline, Mortar-pastel, Insulin syringe, Syringe 5ml and 10ml, Ketamine/Pentobarbitol, Screw cap test tube, H_2SO_4 (2N), NaOH(1N,)Sucrose solution, Homogenizer, Water bath, Vortex, centrifuge and Glucose kit.

Experimental Procedure/Methods

Drying and grinding

The plants selected for this study; *Maytenus emarginata* (Willd.) stem was thoroughly rinsed with distilled water, separated from undesirable materials or plant parts, partially dried by fan aeration and then fully dried in oven at below 40ºC for 2 days. The fully dried stems was then grinded to powdered form and storeed in in the refrigerator at +4ºC for a few days.

Cold extraction

Approximately 100gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 500ml of 80% ethanol, sealed and kept for a period of 2 days with occational shaking and stirring, it was then filtered first by cotton material and twice through Whatman filter paper to obtain a fine filtrate. The filtrate (ethanol extract) obtained was evaporated by Rotary evaporator at 4 to 5 rpm at 65ºC and then dried in the freeze drier and preserve at 4ºC.

Preliminary Phytochemical Screening

The extract was subjectedto preliminary phytochemical screening to test the presence of alkaloids,carbohydrates and reducing sugars, glycosides, proteins and aminoacids, steroids and triterpenoids, phenolic compounds and tannins,flavonoids, fixed oils and fats, volatile oils, gums and mucilages. [15]

All the chemicals and solventsused for analysis were obtained from SD-fine Chemicals, India and thereagents were freshly prepared.

For testing the presence of alkaloids, carbohydrates and reducingsugars, glycosides, phytosterols, saponins, phenolic compounds, tannins,gums and proteins

Table – 1:Preliminary Phytochemical Screening

S. No.	Phytochemical test	Remarks
1	Carbohydrate	+ Ve
2	Protein	−Ve
3	Tannins	−Ve
3	Alkaloids	−Ve
4	Flavonoids	+ Ve
5	Steroids	+ Ve
6	Triterpenoids	+ Ve
7	Phenols	+ Ve

Antidiabetic Activity

Experimental animals

Mice and Long Evans rats (male and female), weighing 80-200g of either sex are used for this study, which are collected from the animal house of the Department of Pharmacology, from our college. All the animals acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2°C, and 12 hours light dark cycle). The animals were fed with standard diet from and had free access to filtered water.

Assessment of the Effect of Plant Materials on Gastrointestinal Motility:

One of the pivotal tasks of gastrointestinal tract is its ability to organize coordinated transport of luminal content which is perfectly adjusted to the digestive needs of the body. To achieve this gastrointestinal tract exhibits a wide repertoire of motor patterns that are based on spatiotemporal coordination of muscle activity. The gastrointestinal tract is able to monitor caloric density; osmolarity and pH of the luminal content and reacts with the initiation of the appropriate motility pattern.

The fascinating variety of motility patterns is best appreciated by imaging gut motility and transit of luminal content by Video fluoroscopy.

Motility disorders in the gut are major causes and concomitant phenomena of various functional, structural and inflammatory bowel diseases; one of the most prominent examples is irritable bowel disease (IBS).

Procedure:

1. For this experiment, 12 hours fasted mince is taken from the animal house.
2. Distilled water is administered to one mouse and marked it with marker as control mouse.
3. Plan extract is administered to another mouse and marked it as test mouse.
4. Barium sulphate milk is prepared by dissolving 10% (W/V) barium sulphate milk in 0.5% (W/V) sodium carboxymethyl cellulose (Na- CMC) suspension.

5. BaSo4 milk is administered to all mice after 1 hour of administration of test drug.
6. Mice are sacrificed after 15 minutes of administration of the BaSo4 milk.
7. The distance travelled by BaSo4 milk is determined by scale.

Screening for the possible inhibition of carbohydrate absorption by plant material

Chemicals and reagents

Normal saline, 2N H2SO4, 1N NaOH, Sucrose (2.5g/Kg body weight of rat in 5ml deionized water)

Drug: 500mg/Kg body weight of rat

Kits: Glucose kit was used for the determination of Glucose.

III.PROCEDURE

Rats were fasted for 20hours before experiment. Sucrose (2.5g/Kg/5ml, average 443 mg) with or without extract (effective dose of hypoglycemic effect). Each segment was washed out with ice-cold saline (10ml), acidified with H2SO4 (2ml) and centrifuged at 3000rpm for 10minutes. The supernatant thus obtained was boiled for 2hours to hydrolyze the Sucrose and then neutralized with NaOH (approximately 2.5ml). The blood glucose level and the amount of Glucose liberated from residual Sucrose in the gastrointestinal tract were measured by Glucose Oxidase (GOD-PAD) Method. Then the gastrointestinal sucrose content was calculated from the amount of liberated glucose.

Assessment of the effect of plant materials on intestinal sucrose activity

Assessment of conditions

All rats were fasted overnight (12hours) before being tested but still allowed free access to distilled water. Extract is administered orally to experiment group and water to control group.

Mucosa/Tissue Collection

After one hour of drug administration, rats are anesthetized with pentobarbital-Na/ether, the entire length of the small intestine (from pylorus to ileocaecal junction) is carefully removed from the pylorus to the ileocaecal junction. The lumen of the intestine is washed out with 50ml of ice cold saline. Intestine is then placed on ice-cold glass plates over ice and cut longitudinally. The mucosa is isolated bt scrapping with glass microscope slides and homogenized with 10ml of saline for 20seconds at medium speed in a HeidolphDiax 600 homogenizer.

Enzyme activities

Sucrose activity is assessed using the Dahlqvist method with modifications. Twenty (20) µl of mucosal homogenate were added in duplicate to 40 mM sucrose and incubated at 37◦C for 60minutes. The glucose converted from sucrose and total protein (using Lowry's methods) in the homogenate are measured. Disaccharidase activity will be calculated by glucose concentration converted from sucrose as µmol-mg glucose/protein/h.

IV.RESULT

Effect of *Maytenus emarginata* (Willd.)GI Motility

Maytenus emarginata (Willd.) extract showed highly significant effect ($p<0.001$) in GI motility. It showed laxative effect. The absorption in the small intestine was decreased on the ingestion of extract.

Table – 2:*Maytenus emarginata* (Willd.)GI Motility test

SL.No	GROUP	% OF GI MOTILITY±SEM
1	Control	52.160 ± 2.29
2	*Maytenus emarginata*	69.129 ± 1.97***
3	Standard (Bisacodyl)	83.156 ± 0.586

***p<0.001

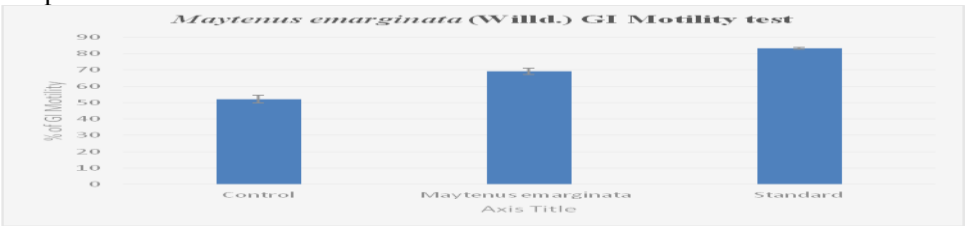

Figure 1: Effect of *Maytenus emarginata* (Willd.) GI Motility

Effect of bark *Maytenus emarginata* on Unabsorbed Sucrose Content in the Gastrointestinal Tract

Upon oral administration of sucrose along with *Maytenus emarginata*(500mg/Kg), significant amount of unabsorbed sucrose was remained in the stomach, upper, middle, and lower intestine at 30 min and 1h. This amount of residual sucrose remained significant in caecum and large intestine till 4h.

Sucrose Absorption Test

Table – 3: Sucrose content in stomach

SL.No.	Groups	Sucrose (mg)			
		30 min	60 min	120 min	240 min
1	Control	53.12 ± 1.6	33.03 ± 3.93	8.49 ± 0.59	1.31 ± 0.31
2	Extract	63.22 ± 0.92*	51.36 ± 1.55*	17.49 ± 2.29	2.54 ± 0.10*

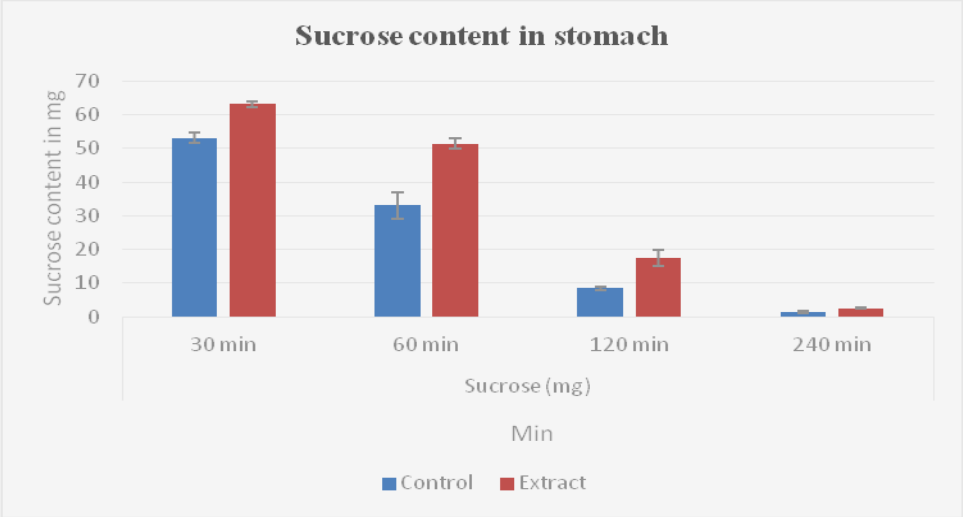

Figure 2: Sucrose content in stomach

Table – 4: Sucrose content in upper intestine

SL.No.	Groups	Sucrose (mg)			
		30 min	60 min	120 min	240 min
1	Control	13.68 ± 0.88	10.67 ± 0.65	3.55 ± 1.07	0.94 ± 0.14
2	Extract	18.05 ± 1.61*	17.30 ± 0.14*	6.29 ± 0.05*	1.66 ± 0.05*

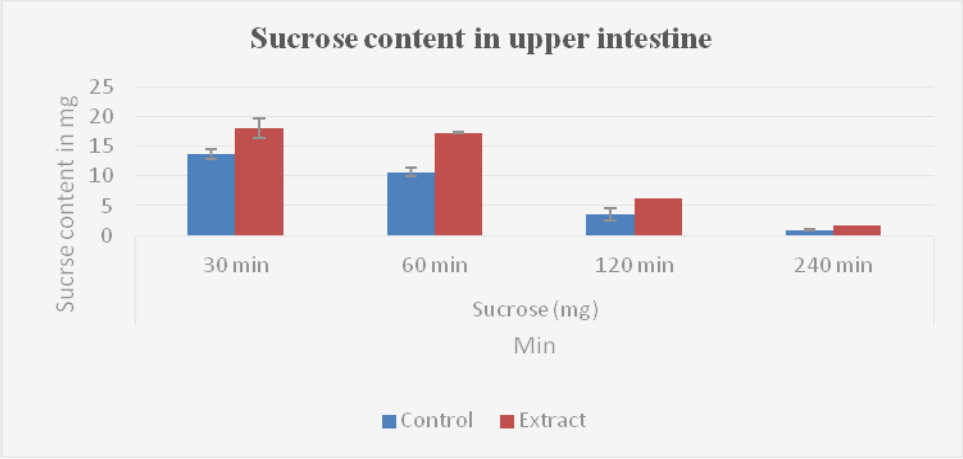

Figure 3: Sucrose content in upper intestine

Table – 5: Sucrose content in middle intestine

SL.No.	Groups	Sucrose (mg)			
		30 min	60 min	120 min	240 min
	Control	19.16 ± 1.94	16.47 ± 0.71	6.98 ± 0.04	1.25 ± 0.07
	Extract	31.48 ± 1.69*	30.73 ± 0.54*	10.29 ± 0.02*	1.96 ± 0.05*

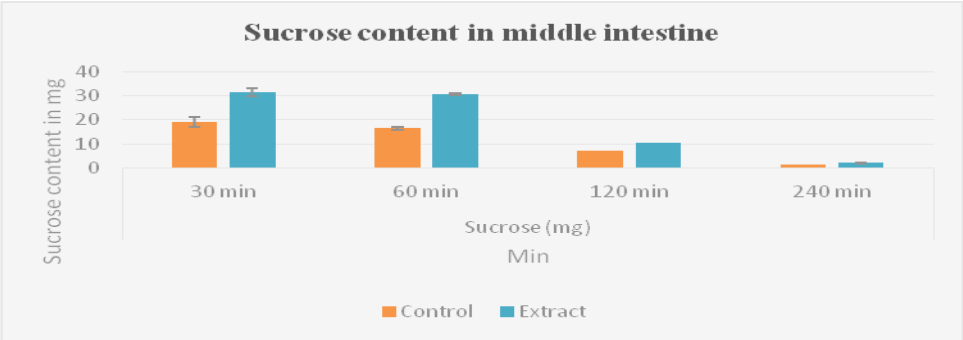

Figure 4: Sucrose content in middle intestine

Table – 6: Sucrose content in lower intestine

SL.No.	Groups	Sucrose (mg)			
		30 min	60 min	120 min	240 min
1	Control	5.56 ± 0.6	3.23 ± 0.72	1.25 ± 0.55	0.97 ± 0.01
2	Extract	5.96 ± 0.15	7.31 ± 0.47*	6.16 ± 0.25*	1.53 ± 0.03*

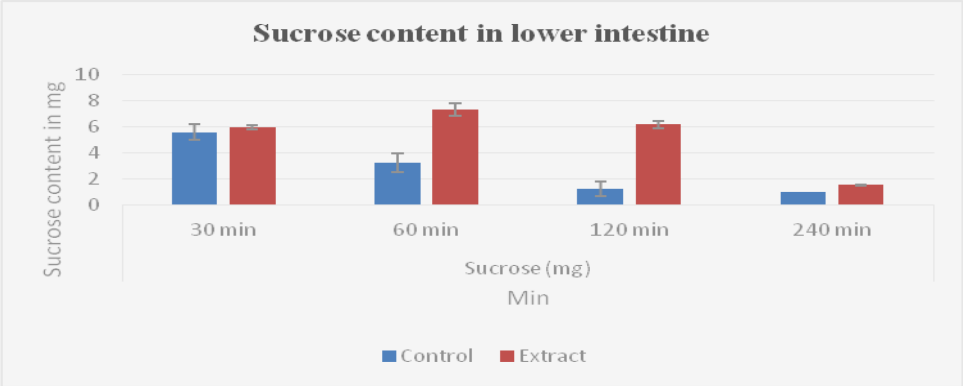

Figure 5: Sucrose content in lower intestine

Table – 7: Sucrose content in Caecum

SL.No.	Groups	Sucrose (mg)			
		30 min	60 min	120 min	240 min
	Control	2.6 ± 0.03	2.0 ± 0.0	1.75 ± 0.03	0.73 ± 0.07
	Extract	5.49 ± 0.02**	6.80 ± 0.64*	5.59 ± 0.07**	1.53 ± 0.05*

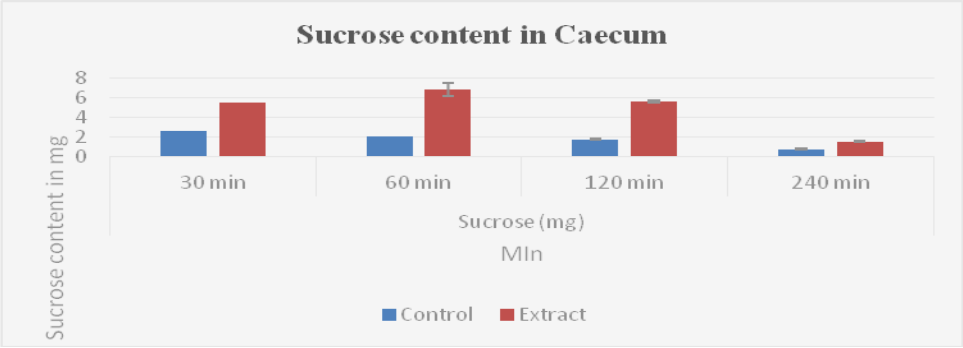

Figure 6: Sucrose content in Caecum

Table – 8: Sucrose content in large intestine

SL.No.	Groups	Sucrose (mg)			
		30 min	60 min	120 min	240 min
1	Control	1.31 ± 0.21	0.93 ± 0.05	0.95 ± 0.14	0.47 ± 0.01
2	Extract	5.38 ± 0.19*	5.69 ± 0.24**	5.39 ± 0.06***	1.01 ± 0.02*

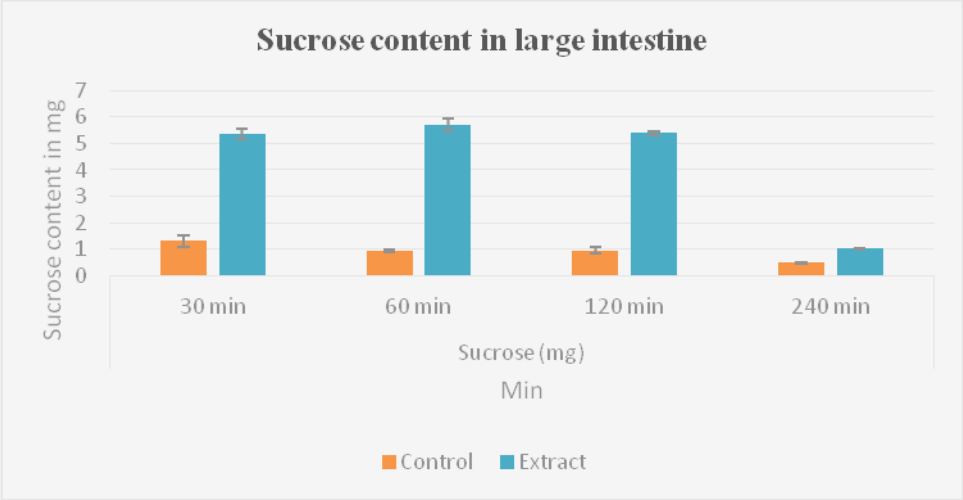

Figure 7: Sucrose content in large intestine

Effects of ethanol extract of *Maytenus emarginata* on gastrointestinal sucrose content after oral sucrose loading in normal rats: Rats were fasted for 20 h before the oral administration of a sucrose solution (2·5 g/kg body weight) with (treated group) or without (control group) ethanol extract of *Maytenus emarginata* (500mg/kg body weight). Values are means and standard deviations represented by vertical bars. This is derived from repeated-measures ANOVA and adjusted using Bonferroni correction.

Effect of *Maytenus emarginata*on Intestinal Disaccharidase Enzyme Activity

*Maytenus emarginata*extract showed highly significant ($p<0.001$) inhibition of disaccharidase enzyme activity.

Table – 9: Disaccharidase activity test of *Maytenus emarginata*

Sl. No.	Group	Disaccharide activity ± SEM (µmol/mg/hr)
1	Control	1.542 ± 0.024
2	Extract	1.045 ± 0.029***
3	Standard	1.063 ± 0.02

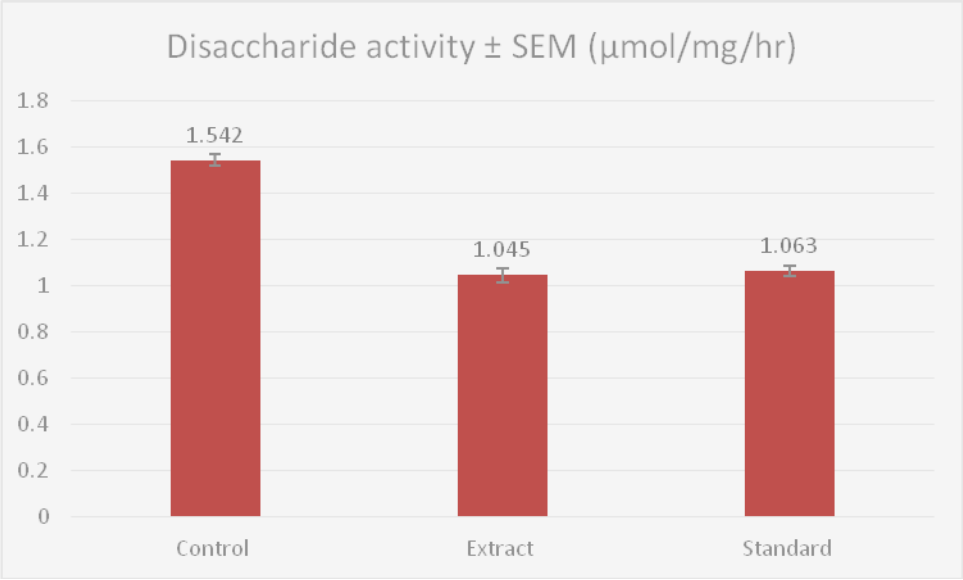

Figure 8: Disaccharidase activity test of *Maytenus emarginata*

Effects of ethanol extract of *Maytenus emarginata* on intestinal disaccharidase activity in normal rats: Rats were fasted for 20 h before the oral administration of ethanol extract of *Maytenus emarginata*(100mg/kg body weight) or water (control). Enzyme activity was determined at 60min. Acarbose (200 mg/Kg) was used as reference control for disaccharidase activity test. Values are means and standard deviations represented by vertical bars (n=12). It significantly decreased ($p<0.001$) disaccharidase enzyme activity (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

V.DISCUSSION

Diabetes and its complications is becoming the third leading cause of death after cancer and cardiovascular diseases. Many serious side effects of insulin therapy and oral hypoglycaemic drugs necessitate the search for newer effective and safer class of compounds to overcome diabetic problems. In recent years, herbal products have started to gain importance as a source of antidiabetic medicines. It has been estimated that more than 1000 plant species are used as folk medicine for treating diabetes though most lack scientific evidence. Our study is directed to evaluate the anti-diabetic property of a methanolic extract of barks of *Maytenus emarginata*on normal rats. Additionally, unpublished, preliminary screening data, of this plant, showed highly promising hypoglycemic activity. Oral treatment with the defatted methanolic leaf extract showed hypoglycemic activity in normal rats. However, the tissue level mechanism of action of *Maytenus emarginata*antidiabetic property is yet to be investigated. According to established studies, the initiator of diabetic tissue damage is the hyperglycaemic states. The cells which are damaged by hyperglycemia cannot maintain a constant internal level of glucose which ultimately results in altered cellular mechanism and long-term changes in cellular macromolecular content. Postprandial glucose spike causes perturbation in endothelial cell function, and increased blood coagulation. An increase in the products of glycosylation is another result of hyperglycaemic states, which significantly influences the development of diabetic induced vascular disease. Thus, management of hyperglycaemic states in diabetes patients is the most important method of diabetes control. Commonly used diabetic drugs follow the basic mechanism of enhancing insulin secretion or enhancing sensitivity to insulin, improving peripheral glucose utilization, inhibiting glucose absorption and intestinal disacharidase enzymes. Through our studies on *Maytenus emarginata*, after using several techniques, we are trying to prove any of the above mentioned mechanism that this plant follows.

Maytenus emarginata showed highly significant effect in GI motility which means it has laxative effect, which results the decreased absorption in small intestine.

Six Segment test showed significantly higher amount of sucrose in stomach, upper, middle and lower intestine in *Maytenus emarginata* administered groups. The latter three part of GI are most important for absorption of nutrients including sugar. Disaccharides in its own form does not get absorbed due to lack to sucrose carriers, as carriers monosaccharaides only are present in the GI tract. Therefore, it is imperative that disaccharides get converted to monosaccharaides first for absorption. Higher sucrose content in the GI Tract clearly reflects a reduced sucrose digestion throughout the GI Tract. This in turn, is shown by a significantly higher concentration of sucrose reaching the large intestine and caecum, which eventually remains unabsorbed and egested with faeces.

In the intestinal disaccharidase activity assay, *Maytenus emarginata*was shown to have reduced the catabolism of sucrose and starch respectively. Since complex carbohydrates and disaccharides have first to be broken down into simpler monosaccharaides, it follows that any inhibition of this catabolic process would retard sugar absorption, which would in turn, be shown as a lower glycemic peak.

Dietary fibers of plant ingredients or powders can often provide a barrier to diffusion caused due to its high viscosity and ability to bind to glucose. Because, dietary fibers are capable of significantly reducing the transit time in GI Tract of ingested food. Reduced transit time is responsible for lesser time available for di-and polysaccharides in the meal to be digested and absorbed.

So, our results can be fully attributed to the significant increase amount of unabsorbed sucrose was remained in 6 different parts of intestine and decrease in disaccharide enzyme activity which validates anti-hyperglycemic activity of *Maytenus emarginata.*

Extract of *Maytenus emarginata*showed significant effect on inhibiting disaccharidase enzyme activity, which does not allow the breakdown of disaccharide to monosaccharide.

The results obtained from both six-segment method and Intestinal Disaccharidase Enzyme Activity test significantly demonstrates, more conclusively, that the methanol extract of *Maytenus emarginata*can be effective in diabetic treatment.

VI.CONCLUSION

Our studies confirm the previous findings showing anti-hyperglycemic action of *Maytenus emarginata.* Additionally, we have elucidated that *Maytenus emarginata*has highly significant capabilities on GI motility and inhibiting absorption of glucose by inhibition of intestinal disaccharidase enzyme. Therefore, its traditional use, as mentioned above is justified and calls for further research, to optimize its anti-diabetic activity.

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