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Research Article

EVALUATION OF ANTI HYPERGLYCEMIC ACTIVITY OF AQUEOUS EXTRACTS OF ALOE VERA (LEAF EXTRACT), AEGLE MARMELOSA (FRUIT EXTRACT), BUTEA MONOSPERMA (FLOWER EXTRACT)

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Abstract: The objective is To know whether the aqueous extracts of *Aloe vera* (leaf extract), *Butea monosperma* (flower extract), *Aegle marmelosa* (fruit extract) are having Antidiabetic activity or not**.** To compare the hyperglycemic effect of Test drugs with that of the Standard Glibenclamide.To compare the potency of the Test drugs with that of Standard Glibenclamide Wistar rats of either sex were administered with alloxan (200 mg/kg/i.p/1wk).The animals under study were kept for fasting for 18hrs and they were subjected to blood glucose analysis. The rats showing FBG greater than 250 mg/dl were selected for the study. These animals were divided into 6 groups of each 6 animals and administered the test drugs (Aloe vera (leaf extract), Butea monosperma (Flower extract) and Aegle marmelosa (fruit extract) orally for 15 days. The blood samples were withdrawn by retro-orbital puncture after 5th, 10th and 15th day of experimentation. The blood glucose levels were estimated by enzymatic method (Trinder's method). From our study we could conclude that out of the three herbal extracts aloe vera had proved to be equipotent as that of Glibenclamide. The study can be extended in formulating the poly herbal formulation which might show the synergistic effect of the three herbal drugs..

Key words: Glibenclamide, Hyperglycemic effect, Antioxidant

INTRODUCTION

In view of the side effects reported with the use of insulin and oral hypoglycemic agents, medicinal plants[1,2] and some active constituents isolated from them are preferred and even recommended by WHO for the treatment of diabetes mellitus[3,4].

MATERIALS AND METHODS [5,6,7]:

DRUGS

The herbal extracts were obtained from chemiliods Pvt. Ltd. Brindavan colony, Vijayawada-10.

Aloe vera (leaf extract) - Batch no: L 9091014.

Butea monosperma (flower extract) - Batch no: L 9091012.

Aegle marmelosa (fruit extract) - Batch no: L 9091013.

Glibenclamide[5] - Batch no: GB 3807, Unichem Pharmaceuticals.

ANIMALS

1. Species/Common name : Wistar rats
2. Age/weight/size : 150-200g
3. Gender : Either sex
4. Number used : 36
5. Source of animals : Animals house of Bapatla college of pharmacy

CHEMICALS USED and PREPARATIONS:

Preparation of 1% sodium CMC: 1gm of sodium CMC was weighed and it was taken in a mortor and pestle and it was triturated for few minutes to get fine powder. Then it was triturated with the addition of small amount of water and made to required volume (100ml), with continuous stirring with water.

Preparation of Citro phosphate buffer p^{H} 4.5: 48.5ml of 0.1M citric acid was mixed with about 40 ml of 0.2M disodium hydrogen phosphate to produce a p^{H} of 4.5 by checking at regular intervals of time with p^{H} meter.

Preparation of citric acid 0.1M: 2.1gm of citric acid was dissolved in sufficient water to produce 100ml.

Preparation of 0.2M disodium hydrogen phosphate: 7.16gm of sodium hydrogen phosphate was dissolved in sufficient water to produce 100ml

Induction of diabetes: After the animals were selected and grouped into six groups, they were fasted for a period of 18hrs. The fasting blood glucose level was then noted. Alloxan was administrated into peritonially by dissolving it in citro-phosphate buffer p^{H}4.5. Alloxan was administered to all the groups except the normal control (Group 1). 200mg/kg of alloxan was given in divided doses for a period of seven days. The elevated blood glucose levels were noted at the end of seventh day.

The selected animals were grouped into 6 groups consisting of 6 animals each

Group 1: Normal control (untreated)

Group 2: Pathogenic diabetic control (Alloxan 150 mg/kg/i.p)

Group 3: Alloxan (200 mg/kg/i.p) + *Butea monosperma* flower extract (500mg/kg/p.o)
Group 4: Alloxan (200 mg/kg/i.p) + *Aegle marmelosa* fruit extract (500mg/kg/p.o)
Group 5: Alloxan (200 mg/kg/i.p) + *Aloe Vera* leaf extract (500mg/kg/p.o)
Group 6: Alloxan (200 mg/kg/i.p) + Glibenclamide (10mg/kg/p.o)

Procedure for withdrawal of blood samples:
Blood samples were withdrawn by retro-orbital puncture after 5th, 10th, and 15th day of experimentation. Every time blood samples were collected in between 9:00 am to 12:00 pm. Each time 0.2ml of blood was withdrawn from right eye. The blood samples were centrifuged for 10 min at 10,000 rpm in order to separate serum for estimating the blood glucose.

Estimation of blood glucose level [8,9,10]:
Analytical method:

The determination of blood glucose is one of the most frequently performed tests in clinical laboratory. Enzymatic methods are usually performed because of their reliability and safety. The glucose kit is based on Trinder's method in which Glucose oxidase and peroxidase enzymes are used along with the chromogen 4-Amino antipyrine and phenol.

Principle
Glucose is oxidized by the enzyme glucose oxidase (GOD) to give D-gluconic acid. Peroxidase (POD) oxidizes phenol which combines with 4-aminoantipyrine to produce a red colored quinoneimine dye. The intensity of the color developed is proportional to theglucose concentration in the sample.

Procedure:
The samples were pipette out into clean and dry tubes labeled as Blank (B), Standard (S), and Test (T), and the reagents were added in the following order.

Table: Procedure for Analytical method of Estimation of blood glucose level

CHEMICALS	BLANK	STANDARD(S)	TEST(T)
GLUCOSE REAGENT(ml)	1.0	1.0	1.0
DISTILLED WATER	0.01	----	----
STANDARD (ml)	----	0.01	----
SERUM	----	----	0.01

The above samples were mixed well and incubated at room temperature for 20 minutes. The Absorbance Test (T), Standard (S), was measured against Blank (B) using spectrophotometer at 505nm.

Equipment used:
1. UV spectrophotometer – ELICO
2. Microcentrifuge – REMI
3. Micropippetes (Tarsens Accupippete) :20-200 micro ml
4. P^H meter – ELICO

Diagnostic agents: Glucose kits were obtained from M/S Excel Diagnostics Pvt.ltd

RESULTS:

Table: 1 Comparison Of Anti Hyperglycemic Effect Of Aloe Vera, Aegle Marmelosa, Butea Monosperma With That Of Standard Drug Glibenclamide

GROUPS	TREATMENT	BLOOD GLUCOSE LEVEL(mg/dl)			
		DAY 0	DAY 5	DAY 10	DAY 15
Diabetic control (Group 2)	Alloxan 200mg/kg/i.p	232.67±2.19	232.7±5.92 b^{***}	231.7±2.39 b^{***}	226.5±3.40 b***
Aloe vera Treated (Group 3)	Alloxan 200mg/kg/i.p+Aloe vera 500mg/kg/p.o	228.8±5.63	155±1.40 a^{***} b^{ns}	118.8±1.51 a^{***} b^{ns}	109.3±2.26 a*** b^{ns}
Butea Monosperma Treated(Group 4)	Alloxan 200mg/kg/i.p+Butea monosperma 500mg/kg/p.o	219.34±2.16	182.2±2.13 a^{***} b^{***}	131.2±1.52 a^{***} b^{***}	119.2±1.37 a^{***} b^{***}
Aegle Marmelosa Treated (Group 5)	Alloxan 200mg/kg/i.p +Aegle marmelosa 500mg/kg/p.o	224.67±5.92	173.8±1.90 a^{***} b^{***}	123.3±1.54 a^{***} b^{ns}	116.2±1.12 a^{***} b^{***}
Glibenclamide Treated (Group 6)	Alloxan 200mg/kg/i.p+Glibenclamide 10mg/kg/p.o	233.34±7.55	161.5±2.14 a^{***}	117.3±1.20 a^{***}	103.2±1.35 a^{***}

The values are represented as mean ± SEM of six observations (n=6) 'a' represents the level of significance when compared to diabetic control 'b' represents the level of significance when compared to that of standard . ANOVA followed by Dunnett's multiple comparison tests was performed
Signifies *($p<0.05$), * *Signifies ($p<0.01$) * * *Signifies ($p<0.001$), Ns – non significant

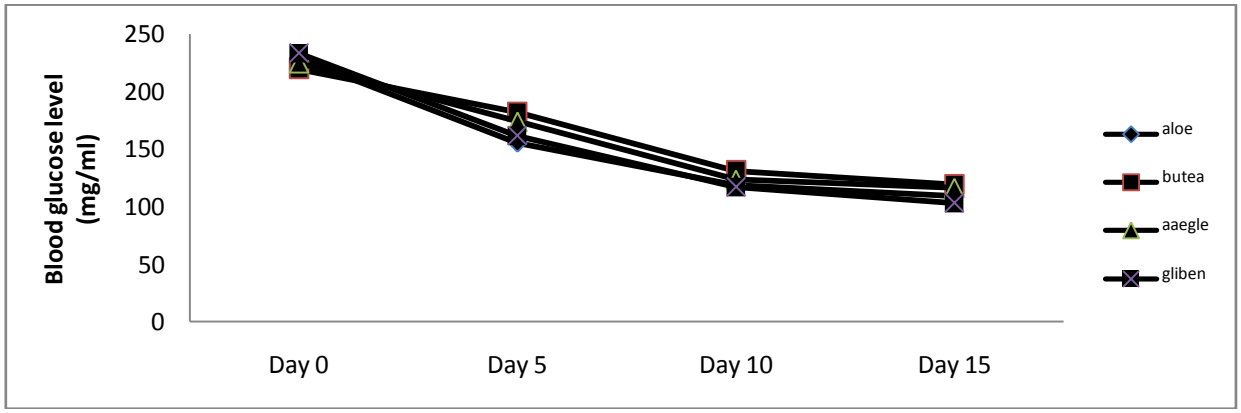

Fig.1 Comparison Of Anti Hyperglycemic Effect Of Aloe Vera, Aegle Marmelosa, Butea Monosperma With That Of Standard Drug Glibenclamide

DISCUSSION

In recent years there has been growing interest in understanding the role of free radicals in many diseases such as cancer, arteriosclerosis, diabetes, ageing and their prevention using anti oxidants. The search for natural anti oxidants has increased over the past few years as the reactive oxygen species (ROS) production and oxidative stress have been shown to play a vital role in a number of disorders. Also the restrictions laid on the use of synthetic anti oxidants have been the important incentive for such research work. These studies are more pertinent with regard to the therapeutic agents of plant origin employed in treating a wide range of Diseases**.**[11, 12, 13, and 14]

Literature confirms the anti oxidant activities of *Aloe vera*, *Aegle marmelosa*, *Butea monosperma* in various in vivo models. The anti oxidant activity[15,16] of *Aloe vera* is due to anthra quinine glycosides and those of *Aegle marmelosa* and *Butea monosperma* are due to flavonoid content. The dose of alloxan 200 mg/kg destroys the beta cells, which ultimately results in hyperglycemia. The increased blood glucose levels in diabetic animals as compared to normal ones may be due to glycogenolysis or gluconeogensis. Treatment of diabetic animals with *Butea monosperma*,

CONCLUSION

The earlier studies support the support the presence of flavonoids which are responsible for the free radical scavenging activity of *Butea monosperma* and *Aegle marmelosa*. From our study we can conclude that due to presence of flavonoids in *Butea monosperma* and *Aegle marmelosa* extracts, they have shown anti hyperglycemic activity. The anti hyperglycemic effect of Aloe vera is due to the presence of anthraquinone glycosides. Out of the herbal extracts *Aloe vera* has proved to be equipotent as that of Glibenclamide.

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