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ANTI-DIABETIC ACTIVITY ON *BREYNIA RETUSA* LEAF IN STREPTOZOTOCIN (STZ) & ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is the most common endocrine disorder that impairs glucose homeostasis resulting in severer diabetic complications. India has today become the diabetic capital of the world with over 20 million diabetes and this number is set to increase to 57 million by 2025. In the present study diabetes was induced in albino rats model with alloxan monohydrate and streptozotocin induced. The effect of chloroform and ethanolic extract of *breynia retusa* leaf was under taken to screen the hypoglycemic activity. The results showed that the ethanolic extract dose of 200mg/kg b.wt and 400mg/kg b.wt has significant antihyperglycemic effect in experimental model of diabetes mellitus.

Key words: *Breynia Retusa*, Alloxan, streptozotocin, Diabetes mellitus, Blood glucose.

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1. INTRODUCTION:

Diabetes mellitus is an endocrine, metabolic disorders caused by relative or an absolute lack of insulin. (Seshiah V. *et al.*, 2016) According to International Diabetes Federation (IDF), worldwide 382 million people were affected by diabetesin 2013 and it is expected to rise to 592 million by 2035. IDF estimates 65 million diabetic patientsin India in 2013and it is expected to cross 109 million by 2030. (IDF Diabetes Atlas 2013)In India diabetic patients are increasing day by day may be because of the change in food pattern, i.e. fast food diet intake and change in lifestyle. (Devi Manickam, *et al.*, 2013)Management of diabetes is a tough task. The medicines used in diabetic treatment are either too costlier or have adverse effects like hypoglycemic coma, insulin resistance, hypersensitivity and metallic taste etc.(Nyemb Nyunai AB, *et al.*,2009)Hence, in the recent years, herbal compounds are gaining popularity in both developed and developing countries because of their natural origin, low adverse effects. (Avinash Patil A, *et al.*, 2013) Ethnobotanical information indicates that around 800 medicinal plants having hypoglycemic or antidiabetic potential. (Sweety Lanjhiyana, *et al.*, 2011)Herbal plants are abundant in India. Hence the search for safer and effective antidiabetic agents has

become the current research area. (Avinash Patil A, *et al.*, 2013)

2. EXPERIMENTAL SECTION

2.1 Material and Methods:

The plant of breynia retusa collected from the chittoor district was authenticated by Dr. K. Madhava Chetty, Ph.D., Department of Botany, S.V University, Tirupathi. Voucher specimen no-0603.the leaves of *breynia retusa* were shade dried after collection of 15 days and was coarsely powdered. The powdered leaf was defatted with petroleum ether and then subjected to continuous hot extraction in soxhlet apparatus with ethyl acetate and ethanol. The extract was filtered through a cotton plug, followed by whatmann filter paper (no.1). The extract was evaporated under reduced pressure using rotovac evaporator at a low temperature (40-60ºc). Phytochemical studies. The various extract of *breynia retusa* were subjected to preliminary phytochemicals and it revealed the presence of flavoinds, terpenoids, steroids , alkaloids, tannins and carbohydrates.

2.2 Animals:

Wistar Albino rats (150-200mg) of either sex were used in this investigation. They were maintained at standard housing condition and fed with commercial diet (Hindustan lever ltd., Bangalore) and provided with water ad libitum during the experiments. The Institutional Animal Ethical Committee permitted the study. Acute toxicity studies acute toxicity study was performed for various extracts of *BREYNIA RETUSA* according to the acute toxic classic methods as per OECD guidelines 14. The animals were kept fasting for overnight providing only water, after which the various extracts were administrated orally at the dose of 2000mg/kg was prepared by dissolving the extract in distilled water and the concentration was adjusted in such a way that it did not exceed 1ml/100g of the rat. The extract was then administrated orally (p.o) and animals were observed for behavioral changes, any toxicity and mortality up to 48 hrs.

2.3 Streptozotocin Induction of Diabetes:

The Streptozotocin-induced (STZ) diabetes induced by STZ administration in rats serves as a validated and reproducible model of retinal hypervascularity in Brown Norway rats (BN). At CBI, this model is induced in male BN rats by STZ administration. Body weights and blood glucose levels were monitored weekly. At 2 and 4 weeks, retinas were examined in vivo using the Micron III retinal imaging microscope (Phoenix Research Laboratories, Pleasanton, CA). Histological examination of the eyes was also performed. The in vivo fluorescein angiograms as captured by the Micron retinal imaging microscope as coupled with histopathologic assessment of the vasculature of the retinal pigmented layer allows for a complete assessment of the micro vascular retinal effects with this model.

2.3.1 Effect of BRLE on antidiabetic activity

2.3.2 Materials:

All the materials used for experiment were of pharmacopoeial grade. Alloxan, Metformin and STZ glibenclamide was purchased from the local supplier. The blood glucose level was determined by using haemoglukotest (20-88R). Glucose strips supplied by M/s Boehringer Mannheim india ltd. Diagnostic kits for the estimation of serum urea,serum creatinine, serum cholesterol and serum protein were purchased from local supplier manufactured by crest biosystems, a division of coral clinical systems,goa.

2.3.3 Experimental Procedure:

Wister albino rats were divided into eleven groups of 6 animals in each group as follows:

Group I	:	Normal control administered with 0.9% sodium chloride (NaCl)
Group II	:	STZ-induced diabetic control administered with 0.9% NaCl;
Group III	:	Diabetic rats administered with Glibenclamide (2.5 mg/kg).
Group IV	:	STZ + BRLCE 200 mg/kg b/wt was given orally after 24hrs, of STZ administered and during next 6days.
Group V	:	STZ + BRLCE 400 mg/kg b/wt was given orally after 24hrs, of STZ administered and during next 6days.
Group VI	:	STZ + BRLAE 200 mg/kg b/wt was given orally after 24hrs, of STZ administered and during next 6days.
Group VII	:	STZ + BRLAE400 mg/kg b/wt was given orally after 24hrs, of STZ administered and during next 6days.

Albino rats of either sex (150-200g) are injected with a single dose of STZ (120mg/kg body weight) dissolved in normal saline by i.p.route animals kept for 48hrs during which food and water is allowed ad libitum. Animals showing fasting blood glucose above 6days of administered orally at dose level of 100 and 250mg/kg body weight.

After the drug treatment, body Weight, insulin was measured on Day 0^{th} and Day 28^{th}. On the 28^{th} day samples were collected from overnight fasted rats by cardiac puncture under mild anesthesia. Serum is separated by centrifuge (3000rpm) under cooling (2-4^{0}C) for ten minutes. The serum biochemical parameters were estimated.

2.4 Allaoxan Induction of Diabetes:

Allaoxan: is a cyclic urea compound, which induces permanent diabetes. It is a highly reactive molecule, which produces free radical damage to beta islet cells & causes cell death. When islets are exposed in vitro to alloxan, it exhibits exceptional beta cell specificity, the other islets cells remaining largely unaffected by both its inhibitory and cytotoxic effects.

2.4.1 Experimental Procedure:

Wister albino rats were divided into eleven groups of 6 animals in each group as follows:

Group I	:	Normal control administered with 0.9% sodium chloride (NaCl)
Group II	:	Alloxan-induced diabetic control administered with0.9% NaCl
Group III	:	Diabetic rats administered with (Metformin).
Group IV	:	Alloxan + BRLCE 200 mg/kg b/wt was given orally after 24hrs, of alloxan administered and during next 6days.
Group V	:	Alloxan + BRLCE 400 mg/kg b/wt was given orally after 24hrs, of alloxan administered and during next 6days.
Group VI	:	Alloxan + BRLAE 200 mg/kg b/wt was given orally after 24hrs, of alloxan administered and during next 6days.
Group VII	:	Alloxan + BRLAE400 mg/kg b/wt was given orally after 24hrs,

of alloxan administered and during next 6days.

Albino rats of either sex (150-200g) are injected with a single dose of alloxan monohydrate (120mg/kg body weight) dissolved in normal saline by i.p.route animals kept for 48hrs during which food and water is allowed ad libitum.animals showing fasting blood glucose above 6days of administered orally at dose level of 200 mg/kg and 400mg/kg body weight.

After the drug treatment, body weight, insulin was measured on Day 0^{th} and Day 28^{th}. On the 28^{th} day samples were collected from overnight fasted rats by cardiac puncture under mild anaesthesia. Serum is separated by centrifuge (3000rpm) under cooling (2-4^{0}C) for ten minutes. The serum biochemical parameters were estimated.

3. RESULTS & DISCUSSION:

3.1 Effect of BRL extracts on STZ induced and Alloxan induced diabetic in rats

Fasting blood glucose level, biochemical parameters like Body weight (g), Insulin (U/L), Total haemoglobin (mg/dL), HbAC (%Hb), Protein (g/dL), Serum urea (mg/dL), Serum Creatinine (mg/dL) of group I to XI animals were estimated quantitatively and tabulated in Table 1 & 2 respectively.

The comparative efficacy of the BRL & BRS extracts tested for their STZ induced & Alloxan induced diabetic in rats, along with the relationship between dose and blood glucose level were depicted in the form of a bar diagram as shown in **Figure 8.11***Fig*- 6.9, 6.10, 6.11, & 6.12, were biochemical parameters depicted in the form of a bar diagram as shown in *Fig*- 6.13, 6.14, 6.15, & 6.16 And body weight in each case were depicted in the form of a bar diagram as shown in *Fig*- 6.17, 6.18, 6.19, & 6.20.

3.1.1 Body Weight

Control group I animals body weight at basal value, 0^{th} and 28^{th} day was 185.23±9.46 & 210.32±2.49 respectively. In diabetic control group II animals having body weight at basal value, 0^{th} and 28^{th} day was 180.67±9.65 & 126.66±6.37 respectively. Diabetic induced group III animals treated with standard drug shows that increase in the body weight at basal value, 0^{th} and 28^{th} day was 188.56±4.16 & 186.39±5.67 respectively. In diabetic induced group IV & V animals treated with BRLCE at dose level of 200 & 400mg/kg b.w, p.o in rats exhibited increase in body weight at basal value, 0^{th} and 28^{th} day was 182.56±4.23 & 168.44±5.32 and 183.52±4.89 & 179.26±4.15 respectively. In diabetic induced group VI & VII animals treated with BRLAE at dose level of 200 & 400mg/kg b.w, p.o in rats exhibited increase in body weight at basal value, 0^{th} and 28^{th} day was 184.16±5.88 & 182.32±3.23 and 185.21±6.04 & 183.54±3.53 respectively.

3.1.2 Insulin (U/L)

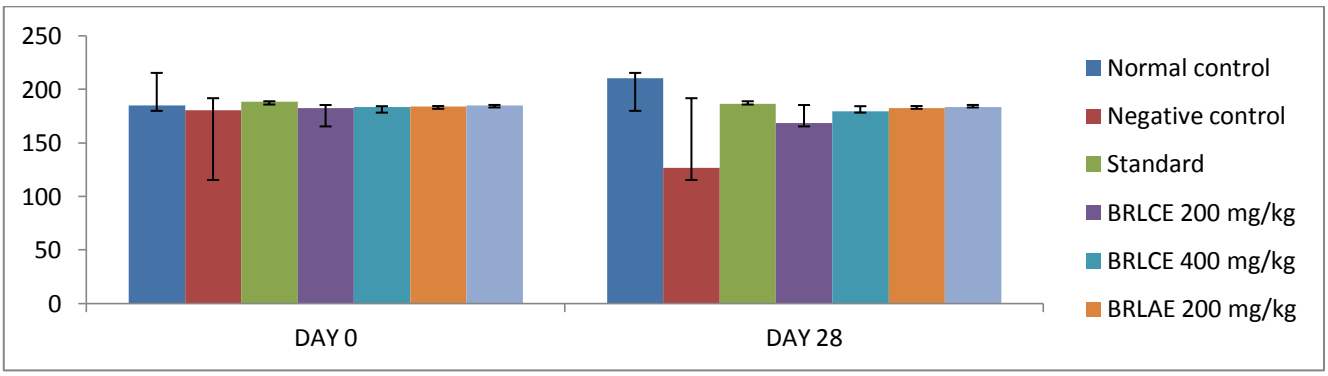
Control group I animals Insulin (U/L) at basal value, 0^{th} and 28^{th} day was 15.47±1.47 & 15.58±1.23 respectively. In diabetic control group II animals having Insulin (U/L) at basal value, 0^{th} and 28^{th} day was 5.78±1.88 & 6.39±0.91 respectively. Diabetic induced group III animals treated with Standard drug shows that increase in the Insulin (U/L) at basal value, 0^{th} and 28^{th} day was 6.43±1.16 & 15.41±1.36 respectively. In diabetic induced group IV & V animals treated with BRLCE at dose level of 200 & 400mg/kg b.w, p.o in rats exhibited increase in Insulin (U/L) at basal value, 0^{th} and 28^{th} day was 6.08±1.88 & 11.96±2.14 and

6.19±1.36 & 13.68±1.24 respectively. In diabetic induced group VI & VII animals treated with BRLAE at dose level of 200 & 400mg/kg b.w, p.o in rats exhibited increase in Insulin (U/L) at basal value, 0^{th} and 28^{th} day was 6.24±1.08 & 14.02±1.02 and 6.36±2.12 & 15.11±1.28 respectively.

3.1.3 Biochemical Parameter in STZ induced

Control group I animals having Total haemoglobin (14.26±0.22), HbAC (5.73±0.85), Protein (7.18±0.67), Serum urea (24.89±3.58), Serum Creatinine (0.29±0.05). In diabetic control group II animals produced elevated levels of serum biochemical parameters as follow Total haemoglobin (10.46±1.32), HbAC (12.62±1.19), Protein (5.47±0.63), Serum urea (67.72±3.24), Serum Creatinine (1.09±0.03). In group III animals treated with standard drug (2.5 mg/kg) in diabetic induced rat's shows that reduction of serum biochemical parameters like Total haemoglobin (14.20±1.02), HbAC (6.68±0.61), Protein (7.54±0.67), Serum urea (27.44±2.85), Serum Creatinine (0.32±0.02).In group IV & V animals treated BRLCE at dose level of 200 mg/kg & 400 mg/kg b.w, p.o in diabetic induced rats exhibited a significant reduction of Total haemoglobin (12.78±1.75 & 13.67 ±1.51), HbAC (8.12± 1.09 & 7.23±0.99), Protein (5.97±0.65 & 6.68±0.62), Serum urea (51.82±2.48* & 42.45± 3.33), Serum Creatinine (0.79±0.03 & 0.48±0.03) respectively. In group VI & VII animals treated with BRLAE at dose level of 200 mg/kg & 400 mg/kg b.w, p.o in diabetic induced rats exhibited a significant reduction of Total haemoglobin (14.02±1.22 & 14.15±1.26), HbAC (7.06±0.93 & 6.71±0.97), Protein (7.43±0.77 & 7.52±0.81), Serum urea (31.14±4.19 & 28.22± 4.27), Serum Creatinine (0.39±0.02 &0.34±0.03) respectively.

BODY WEIGHT



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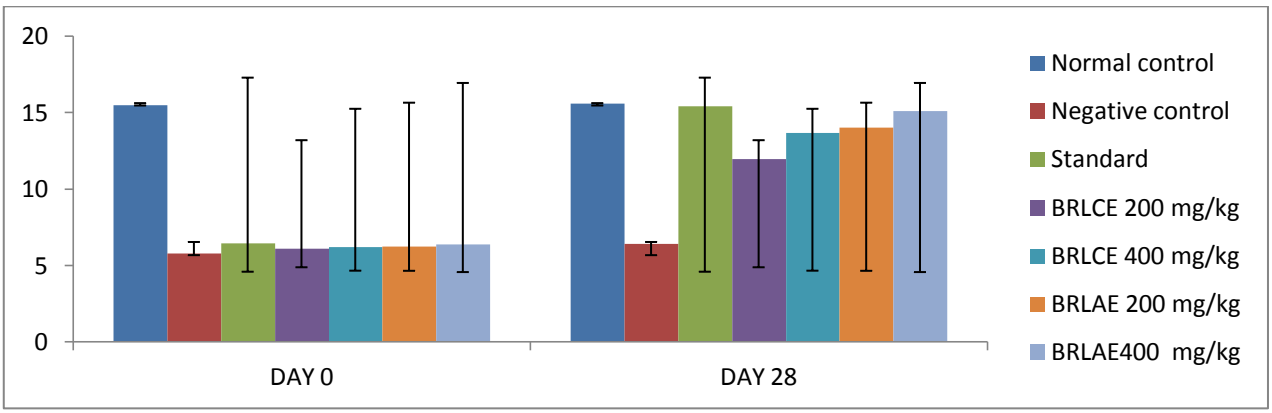
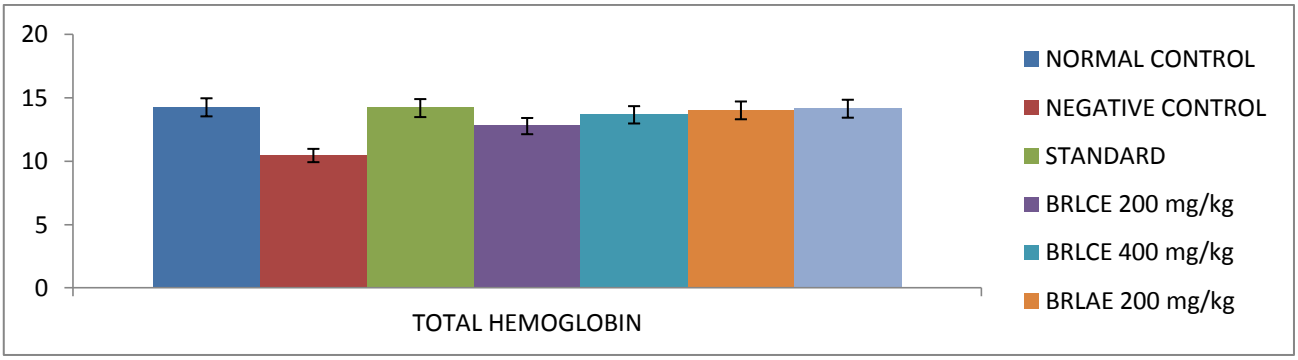


TOTAL HEMOGLOBIN



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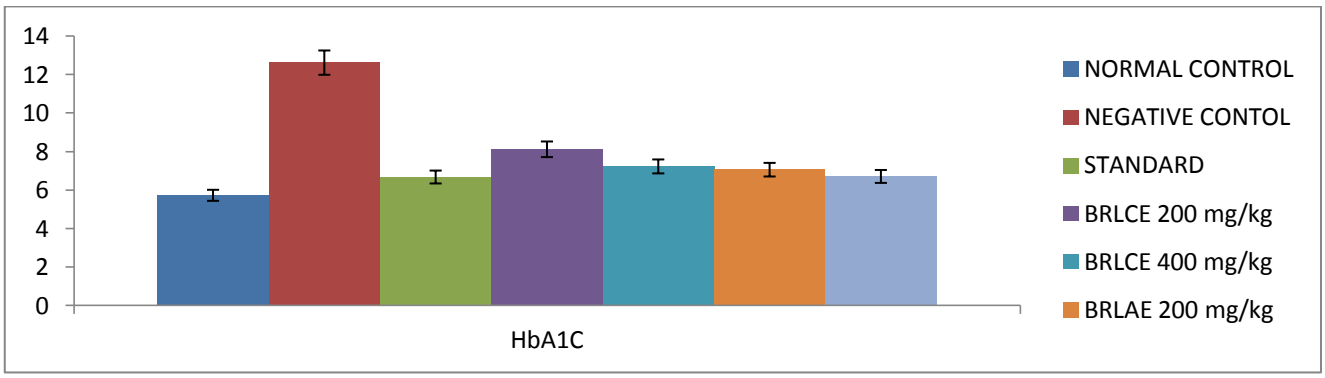

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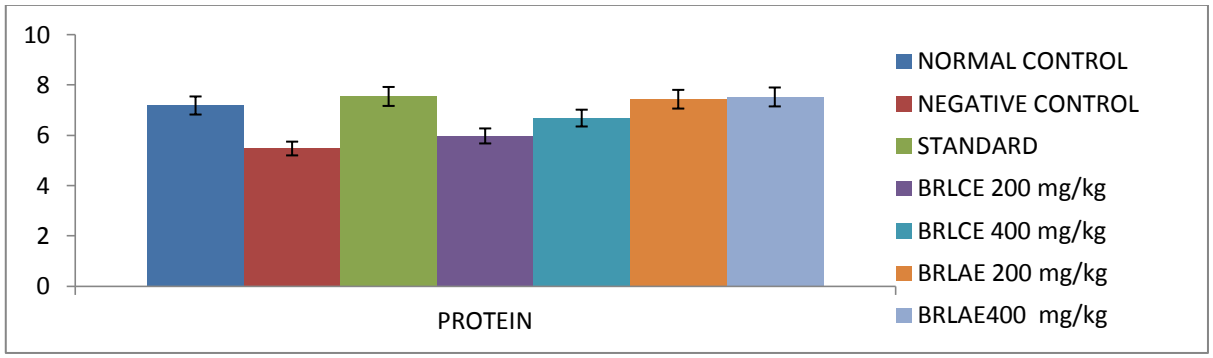

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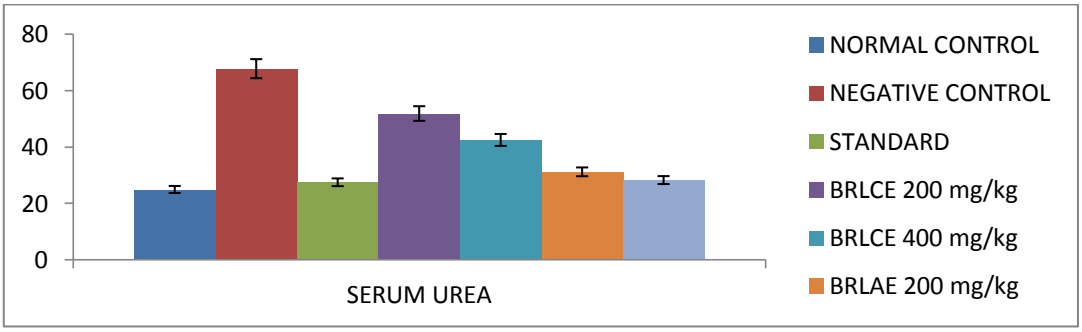
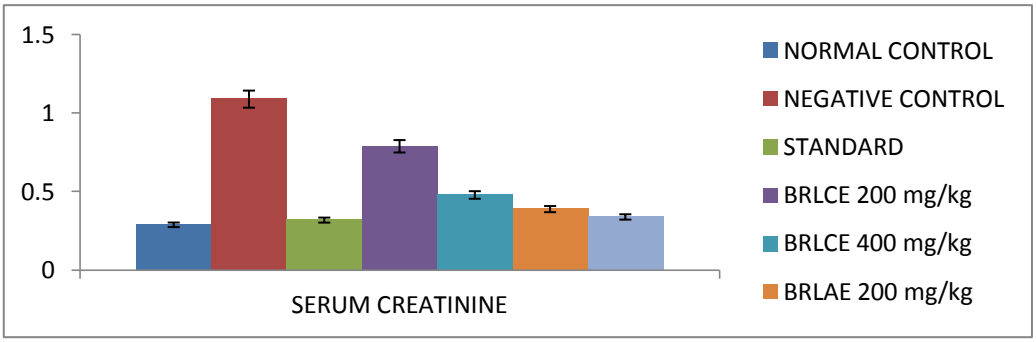


SERUM CREATININE



Biochemical Parameter (Alloxan)

Control group I animals having Blood glucose (119.7±1.33), Triglycerides (92.8± 1.53), Cholesterol (107.7± 1.54), HDL (41.8±0.94), SGOT (92.17± 1.07), SGPT (86±1.29). In diabetic control group II animals produced elevated levels of biochemical parameters as follow Blood glucose (269±1.16), Triglycerides (168.8±1.97), Cholesterol (223.5±2.44), HDL (23.6±1.62), SGOT (129.5±1.74), and SGPT (120.7±1.16). In group III animals treated with standard drug in diabetic induced rat's shows that reduction of biochemical parameters like Blood glucose (121.3±1.25), Triglycerides(94.6±1.43), Cholesterol(109.7±1.88), HDL (44±0.89), SGOT (94± 1.18), SGPT (88.3± 0.88).In group IV & V animals treated BRLCE at dose level of 200 mg/kg & 400 mg/kg b.w, p.o in diabetic induced rats exhibited a significant reduction of Blood glucose (196.4±1.75 & 175.2±1.22), Triglycerides(151.2±1.42 & 143.4±1.68), Cholesterol (178.2±1.65 & 169.1±1.88), HDL (28.5±1.56 & 33.3±1.08), SGOT (123.5±1.87 & 115.7±1.25), SGPT(115.2±0.94 & 111.4±1.29) respectively. In group VI & VII animals treated with BRLAE at dose level of 200 mg/kg & 400 mg/kg b.w, p.o in diabetic induced rats exhibited a significant reduction of Blood glucose (151.8±1.67 & 132.3±1.08), Triglycerides (134.5±1.51 & 125.4±1.60), Cholesterol (156.7±1.59 & 139.2±1.46), HDL (37.9±1.42 & 42.6±0.98), SGOT (107.1±1.34 & 98.3±1.12), SGPT (106.8±1.06 & 101.7±1.92) respectively.

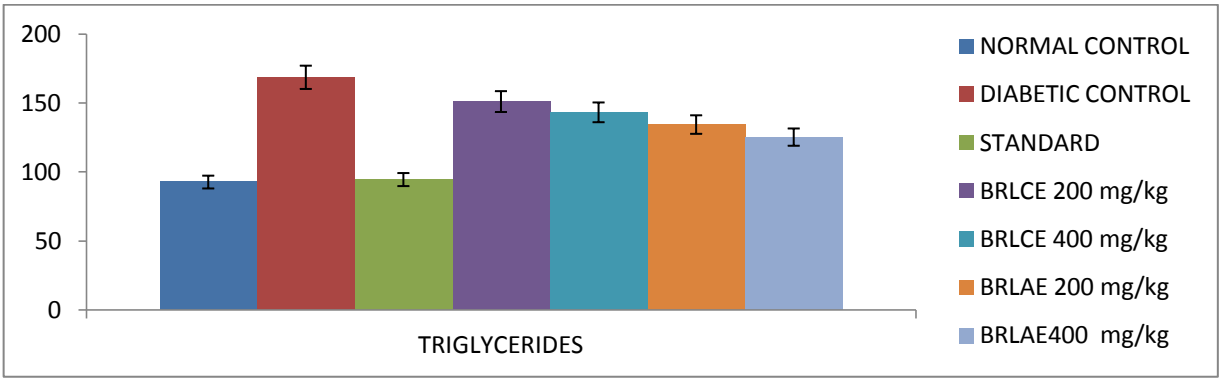
Effect of BRL and BRS extracts on Alloxan induced diabetes

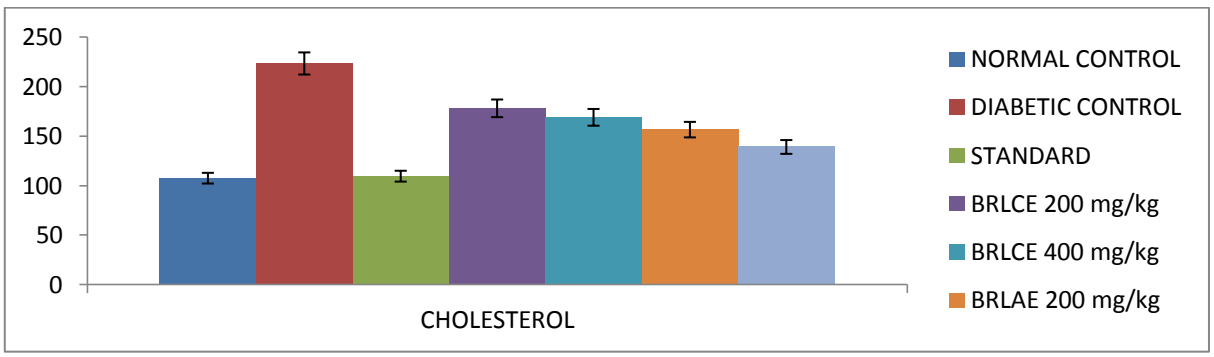
Groups	Blood glucose	Triglycerides	Cholesterol	HDL	SGOT	SGPT
Normal control	119.7±1.33	92.8± 1.53	107.7± 1.54	41.8±0.94	92.17± 1.07	86±1.29
Diabetic control	269±1.16 **	168.8±1.97**	223.5±2.44**	23.6±1.62	129.5±1.74**	120.7±1.16**
Standard (Metformin)	121.3±1.25**	94.6±1.43**	109.7±1.88**	44±0.89**	94± 1.18**	88.3± 0.88 **
BRLCE 200 mg/kg	196.4±1.75**	151.2±1.42**	178.2±1.65**	28.5±1.56**	123.5±1.87**	115.2±0.94**
BRLCE 400 mg/kg	175.2±1.22**	143.4±1.68**	169.1±1.88**	33.3±1.08**	115.7±1.25**	111.4±1.29**

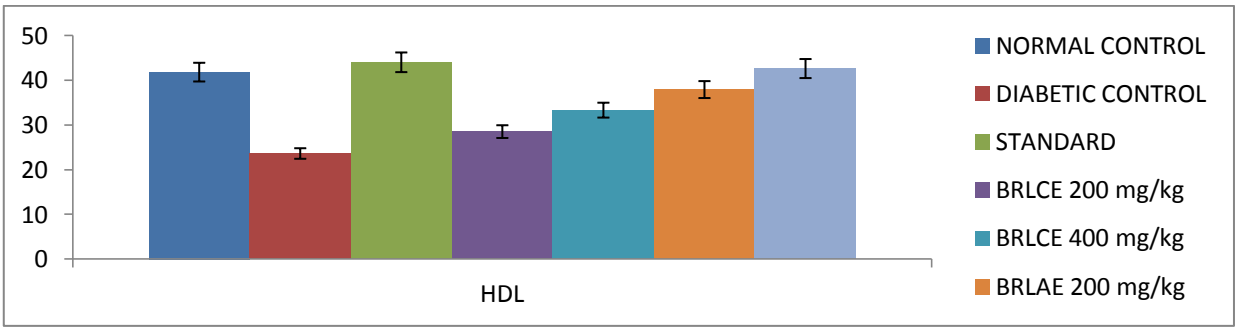
BRLAE 200 mg/kg	151.8±1.67**	134.5±1.51**	156.7±1.59**	37.9±1.42**	107.1±1.34**	106.8±1.06**
BRLAE 400 mg/kg	132.3±1.08**	125.4±1.60**	139.2±1.46**	42.6±0.98**	98.3±1.12**	101.7±1.92**

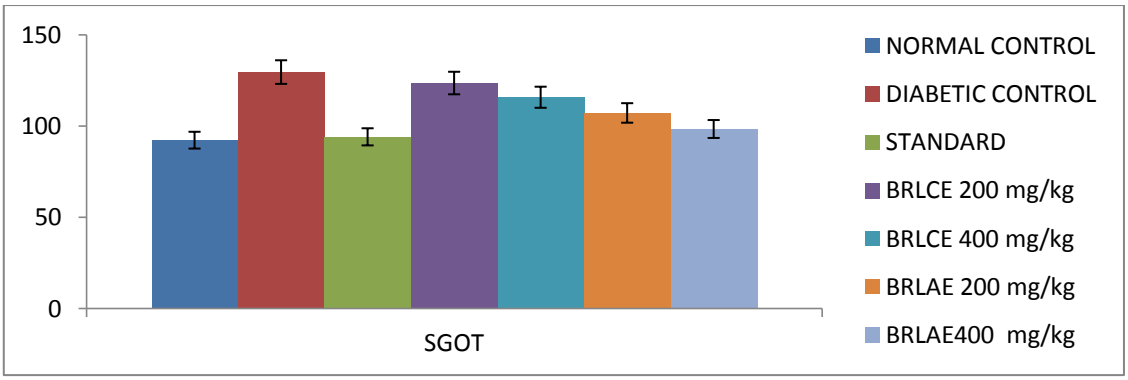
*P< 0.05 Significant, **P< 0.001 highly significant.

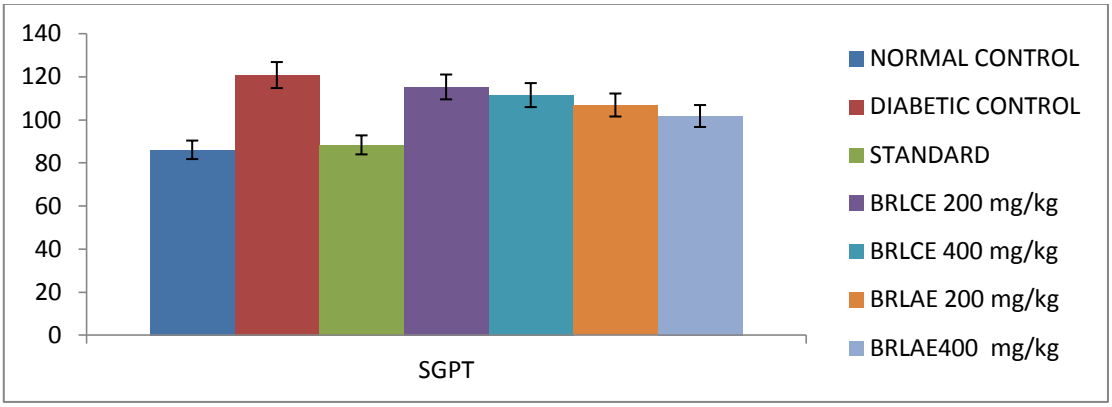
Effect of BRL & BRS extracts on Blood Glucose











Alloxan is one of the usual substances used for the induction diabetes mellitus. Alloxan has a destructive effect on the beta cells of pancreas. Alloxan causes a massive reduction in insulin release by the destruction of β cell of the islets of langerhans, there by inducing hyperglycaemia.

BRLCE & BRLAE and BRSCE & BRSAE at different concentration were studied for anti- diabetic activity. The study was performed using STZ induced and alloxan induced diabetic models in rats for 7days and the effects were compared with standard drug. . Oral administration of BRLCE & BRLAE and BRSCE & BRSAE at different concentrations of STZ induced and alloxananised rats significantly decreased blood glucose levels, body weight, biochemical parameters like serum urea, serum creatinine, and serum cholesterol and serum protein. These extracts contain myriad number of compounds like tannins, phenolic compounds and alkaloids, among them methyl tiglate in both extracts may be responsible for anti-diabetic activity.

This results in present study indicate that BRL & BRS extracts was found to reduce the glucose level, serum urea, serum creatinine, serum cholesterol and increase the serum protein and body when compared to that of diabetic induced animals.

The anti-diabetic activity of BRLAE at 200mg/kg is more significant than that of BRLCE at 200mg/kg. The anti-diabetic activity of BRLE is more significant than that of BRSE.

CONCLUSION:

From this study, we can state that the ethanolic extract of *Breynia Retusa* has beneficial effects on blood glucose level as well as improving hyperlipidemia and other metabolic aberrations. It has the potential to impart therapeutic effects in diabetes.

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