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EFFECT OF BREYNIA RHAMNOIDES LEAVES EXTRACTION HIGH FRUCTOSE DIET INDUCED C57BL/6J OB/OB DIABETIC MICE

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ABSTRACT

The objective of the present study was to investigate anti-diabetic and nephroprotective and cardioprotective activity of *Breynia rhamnoides* leaves extract, using High Fructose induced diabetic C57BL/6J*ob/ob* mice as model for clinical type-2 diabetic. At a regular interval of experimental protocol blood glucose, urinary creatinine, total proteins, insulin resistance, total β-cell count, LDL, HDL, VLDL, and organs to body weight ratio were studied. The histo-pathological study was carried out by High Fructose induced diabetic and anti-diabetic rats pancreas. Statistical analysis of the results shown that in High Fructose induced diabetic rats chloroform and alcohol extracts of *Breynia rhamnoides* leaves at 40, 80, 160 and 200 mg/kg doses. *Breynia rhamnoides* leaves extract improved renal creatinine clearance, decreased the LDL, VLDL, increased the HDL and reduce renal total protein loss demonstrating nephroprotective and cardioprotective properties. The organ to body weight ratio studies carried out on last day, shown pancreas and liver specific effects of *Breynia rhamnoides* leaves, these results were also supported by histo-pathological studies. We conclude from the present study that *Breynia rhamnoides* alcoholic extract and *Breynia rhamnoides* chloroform extract long-term treatment may be beneficial in the management of diabetes.

KEYWORDS

Breynia rhamnoides (BRR), high fructose diet, antihyperglycemic, C57BL/6J*ob/ob* mice.

INTRODUCTION

The metabolic syndrome characterized by insulin resistance, dyslipidemia, and hypertension is associated with increased risk of type-2 diabetes and coronary heart disease, resulting in reduced quality of life and increased risk of mortality and morbidity [1]. Diabetes is associated with sustained high glucose content in the blood beyond a certain level that leads to long term damage, dysfunction and failure of various organs including eyes, kidney, nerves, heart and blood vessels. Insulin plays a central role in the regulation of glucose homeostasis and acts in a coordinated fashion on cellular events that include the regulation of ion and amino acid uptake, protein synthesis and degradation, gene transcription and mRNA turnover, and cellular growth and differentiation [2]. An impairment of insulin action (insulin resistance) is involved in many diseases, including noninsulin dependent diabetes, obesity, hypertension and cardiovascular disease [3]. The development of new treatment modalities requires animal models that mimic the range of pathophysiogical changes seen in diabetic humans [4]. The most common is the C57BL/6J ob/ob diabetic mice model because of the complications of diabetes induced by streptozotocin. However, streptozotocin induces type-1 diabetes and experimental results from this model may be relevant only to a small proportion of diabetic patients. Type-2 diabetes is associated with complications such as hypertension, endothelial damage, cardiac hypertrophy, inflammation, atherosclerosis, ventricular contractile dysfunction, fibrosis, retinopathy, neuropathy and nephropathy. Diet induced models of type-2 diabetes, C57BL/6J ob/ob diabetic mice than the streptozotocin-induced model of type-1, may serve as a better vehicle to investigate possible interventions for these complications [5]. Both human and animal studies have shown that fructose is a highly lipogenic nutrient that contributes to insulin resistance, metabolic defects and development of a pre-diabetic or diabetic state [6]. High fructose diet in C57BL/6J ob/ob diabetic mice (≥60% of the diet) has been used to induce cardiovascular symptoms such as hypertension, hypertriglyceridemia, increased collagen deposition in the heart and kidneys associated with increased oxidant concentC57BL/6J ob/ob diabetic mice ion and decreased antioxidant defences [7–8]. These features are almost identical to clinical type-2 diabetes. Hence high fructose diet-induced diabetes in C57BL/6J ob/ob diabetic mice s is used to evaluate ant-diabetic drugs. This study was designed to examine the possibility of the antidiabetic effect of *Breynia rhamnoides* leaves (Euphorbiaceae) in diabetic C57/BL6J *ob/ob* mice; which was considered a good model for type 2 diabetes as it displays many of the characteristics of the human diseases including hyperglycemia, insulin resistance and progressive obesity [9]. In humans, the occurrence of type 2 (non-insulin dependent) diabetes mellitus has been related to a strong

genetic influence. In mice, the autosomal recessive diabetes (*db*) mutation results in metabolic changes similar to those observed in type-2 diabetes in humans. The relative diabetes susceptibility observed among certain inbred strains carrying either the *db* mutation on chromosome 4, or the obesity (*ob*) mutation on chromosome 6, provides evidence of genetic differences. While the nature of this genetic influence is unknown, both *db/db* and *ob/ob* mice exhibit profound resistance to insulin [10]. There is increasing evidence that indicates that oxidative stress produced under hyperglycemia can cause, or lead to, insulin resistance and diabetic complications [11]. Moreover, several studies have shown that antioxidants amelioC57BL/6J ob/ob diabetic mice number of altered physiological and metabolic parameters that occur as a result of type-2 diabetes [12, 13]. The *Breynia rhamnoides(BRR)* leaves extract, which is reported to have a potent antioxidant property and used traditionally in Indian system of medicine to treat diabetes, was selected to screen for possible antidiabetic activity in high fructose diet-induced and C57BL/6J *ob/ob* diabetic mice. Also the *Breynia rhamnoides (BRR)* leaves extract are reported to contain taraxerol and taraxerone as the main constituents to have both antioxidant and antidiabetic properties [13]. However, there is no available evidence of such an effect of *Breynia rhamnoides* leaves extract in type-2 diabetes or an insulin resistant animal model. Based on these profiles of *Breynia rhamnoides(BRR)* leaves, work was undertaken to screen the leaves of the plant for its antidiabetic activity in type 2 diabetes.

MATERIAL AND METHODS

Plant Material Collection and Authentication

In the present study, the *Breynia rhamnoides(BRR)* leaves extract collected from Bijapur District, Karnataka, India. The plant authenticated by taxonomist and consultant Dr. M.S Patil, HOD Botanical department BLDEA'S College of science Bijapur, Karnataka, India. The leaves were dried under room temperature until free from the moisture. Finally, the dried leaves were subjected to get coarse powder and then passed through sieve no. 44 to get uniform powder. The sieved powder was stored in air tight, high-density polyethylene containers before extraction.

Drug and Chemicals

Fructose diet is purchase from Rajesh chemicals Pune. Glibenclamide was obtained as gift sample from Mumbai. All chemicals used in this study AR grade.

Plant Preparation

The powdered leaves *Breynia rhamnoides(BRR)* was subjected to hot continuous, successive extraction (soxhlet) 24 hours cycle with petroleum ether, chloroform and methanol (50-55ºC), then the solvent was distilled off, and excess solvent completely removed by using a rotary flash evaporator or to get chocolate colored semisolid extract. The obtained semisolid mass completely dried in mini lyophilizer. Its percentage yield calculated in terms of air-dried weight of plant material. The crude drug defatted with petroleum ether. The obtained extracts subjected to evaluate for its anti-diabetic activity. The percentage yields of chloroform and methanol extracts are (0.75% and 2.11%) respectively.

Animal Selection

C57BL/6J mice 10–14 weeks old (weighing 50–60 g)) were used for the high fructose diet model. C57BL/6J mice were procured from the National Institute of Nutrition, Hyderabad and used as the diabetic *ob/ob* mice model. For toxicity evaluation mice were procured from HSK College of Pharmacy. Department of Pharmacology (Bagalkot, Karnataka, India).The C57BL/6J ob/ob diabetic mice and mice were housed in polypropylene cages and maintained under suitable nutritional and environmental (12-hour light–12-hour dark cycle: 25 ± 3°C and 35%–60% humidity) conditions throughout the experiment. All the experimental protocols were approved by the institutional animals' ethics committee (HSKCP/IAEC. Clear 2004-05. Dated: 27/3/20013), HSK College of Pharmacy, (Bagalkot, Karnataka, India).

Diagnostic Kits

Glucose Reagent Kit (Aspen Labo Pvt.Ltd, Delhi.), Creatinine Reagent Kit (Aspen Laborites Pvt. Ltd, Delhi), HDL Cholesterol Kit (MR), Protein- CSF kit (Biolab- diagnostics (I) PVT.LTD, Tarapur Boisar, Maharashtra.

Photochemical Analysis

The various extracts of roots of *Breynia rhamnoides* eaves extract were subjected to the following test for the identification of its various active constituents by standard methods. Carbohydrates were identified by Molisch's test, Fehlings test and Benedict's test. Alkaloids by Dragendorff's test, Mayer's test and Hager's test. Glycosides by Legal Test, Baljet test, Keller Killiani test, Borntrager's test, Flavonoids by Shinoda's test and Sodium hydroxide test, Steroids by Libermann-Burchard test, Salkowski's test, Fixed oils and fats by Saponification [6].

Instruments

Research Centrifuge (REMI-24), Borosil Soxhlet Extractor, Auto-Analyzer (Star 21 plus), Research Microscope (Metzer), Afcoset Digital Balance (E-R-180 A) and Mini Lyotrap (LTE Scientific LTD, Great Britain).

Induction of Experimental Diabetics

The high fructose diet contain vegetable starch (527 g/kg diet), fat as vegetable oil (35 g/kg diet), animal protein (220 g/kg diet), and addition of sodium salt, fiber, mineral and vitamin mix used in the experimental diets. The free access to food and tap water, were maintained under standard conditions (20-22^{o}C and a 12-h light/dark cycle) and were weighed weekly.

High Fructose Diet Induced Diabetic C57bl/6j Ob/Ob Diabetic Mice Model

Normal received a tween 80 (2%) with animal feed diet, Positive control received (standard drug) glibenclamide (5 mg/kg po), test received *Breynia rhamnoides* chloroformic and methanolic leaves extract 40, 80, 160 and 200mg/kg, All the test, standard and control animals received standard diet along with drug treatment for 21 days.For biochemical parameter study blood was collected by retro-orbital puncture 1, 3, 7, 11, 15, 17 days.

Experimental Grouping of Animals

Different groups of rats were used to study the effect of methanolic and chloroformic leaves extracts of *Breynia rhamnoides* . The rats were divided into six groups out of this four group of chloroformic extract and four group of methanolic extract, normal, and negative and positive control group are common, each group consisting of six rats:

Group I: The rats received Normal Saline. These animals serve as normal controls (normal).
Group II: Received Glibenclamide 5mg/kg for 21 days and served as positive control (std).
Group II: Received a high fructose diet orally and served as negative control (control).
Group III: Received the *Breynia rhamnoides* methanolic extract (BRRME) 40mg/kg for 21 days and served as Test I.
Group IV: Received the *Breynia rhamnoides s* methanolic extract (BRRME) 80mg/kg for 21 days and served as Test II.
Group V: Received the *Breynia rhamnoides* methanolic extract (BRRME) 160mg/kg for 21 days and served as Test III.
Group VI: Received the *Breynia rhamnoides* methanolic extract (BRRME) 200mg/kg for 21 days and served as Test IV.

Group VII: Received the *Breynia rhamnoides* chloroformic extract (BRRCE) 40mg/kg for 21 days and served as Test V.

Group VIII: Received the *Breynia rhamnoides* chloroformic extract (BRRCE) 80mg/kg for 21 days and served as Test VI.

Group IX: Received the *Breynia rhamnoides* chloroformic extract (BRRCE) 160mg/kg for 21 days and served as Test VII.

Group X: Received the *Breynia rhamnoides s* chloroformic extract (BRRCE) 200mg/kg for 21 days and served as Test VIII.

Estimation of Serum and Urine Bio-Chemical Parameters

Serum Glucose: Blood samples (2 ml) were collected from the C57BL/6J ob/ob diabetic mice by retro-orbital puncture under mild ether anesthesia on the 1st, 4th, 7th, 10th, 13th, and 21st day of the study. The serum was immediately seeped by centrifugation and the glucose level was measured by GOD/POD method using glucose reagent kit and by auto-analyzer.

Urine Creatinine: Creatinine level in urine was estimated by alkaline picric acid method using creatinine kit by auto- analyzer**.**

Urine Total Proteins: Concent of total proteins in urine was estimated auto & manual method using Protein-CSF kit by auto-analyzer**.**

Estimation of HDL

The blood HDL level will be estimated by Cholesterol and HDL Cholesterol Kit (MR) by auto- analyzer.

Estimation of Triglyceride: The blood Triglyceride level will be estimated by GPO Method by auto- analyzer.

Organ to Body Weight Ratio: At the end of the study, animals were sacrificed and adrenal glands, kidneys, liver, heart and pancreas were isolated and weighed in wet condition to measure organ to body weight ratio.

Statistical analysis: Results were expressed as mean blood glucose levels ± S.E.M. (standard error of the mean). Data were analyzed by using student's t-test. P values less than 0.05 was considered to be statistically significant.

RESULTS

Effect of *Xyliadolabriformis*leaves Extract in High Fructose Diet-Induced Change In Serum Glucose and Urine Biochemical Parameters (Mg/Dl).

Fructose feeding significantly increased serum glucose when compared to normal C57BL/6J ob/ob diabetic mice (201.75 mg/dl, in methanolic extract [Table 1] and 183.65 mg/dl in chloroformic extract [Table no-2]. Administration of C57BL/6J ob/ob diabetic mice chloroform and methanolic extract of *Breynia rhamnoides* leaves extract (40,80,160 and 200 mg/kg) along standard drug Glibenclamide shows significantly (*P* □0.001) reduced the serum glucose values in a dose dependent manner during 21 days of the experimental study when compared to the control group fed high fructose diet (Table 1).

Effect of *Breynia Rhamnoides* Leaves Extract in High Fructose Diet-Induced Change In Serum Triglycerides and Cholesterol Biochemical Parameter (G/Dl).

Hypertriglyceridemia and hypercholesterolemia are common features in animal models of insulin resistance induced by a high fructose diet. Increased levels of triglycerides and cholesterol are the main predictors and/or causative agents for inducing the insulin resistance in type-2 diabetes. The triglyceride and cholesterol levels were significantly.

Effect of *Breynia rhamnoides* leaves extract in high fructose diet-induced change in serum total protein biochemical parameter.

The total protein in the urine of the *Breynia rhamnoides* leaves extract (40,80,160,200 mg/kg)-treated group of animals reduced significantly (11.23 to 6.46 g/dL, *P* □0.05) (Table-4), suggesting partly the nephroprotective activity of the drug. Low dose of *Breynia rhamnoides* leaves extract (40 mg/kg) was not significant in showing nephroprotective activity.

Effect of *Breynia rhamnoides* chloroform& methanolic leaves extracts on serum creatinine & total protein (mg/dl) in high fructose diet induced diabetic C57BL/6J ob/ob diabetic mice.

Significant ($p<0.01$) change in renal creatinine clearance was observed in *Breynia rhamnoides* chloroform & methanolic leaves extracts and glibenclamide and treatment groups. When high fructose diet fed C57BL/6J ob/ob diabetic mice s treated continuously at all doses for 21 days has significantly ($p<0.01$) increased the renal performance when compared to control group.

Effect of *Breynia rhamnoides* leaves extract on high fructose diet induced change in Organs to body weight C57BL/6J ob/ob diabetic mice io in (mg/gm).

Feedinghigh fructose diet significantly increase ($p<0.05$) the weight of the liver kidney, pancreas and heart to body weight C57BL/6J ob/ob diabetic mice io. Continuous daily treatment with glibenclamide and *Breynia rhamnoides* leaves extracts significantly ($p<0.05$) decreased high fructose diet induced increased kidney, liver, pancreas and heart to body weight C57BL/6J ob/ob diabetic mice.

Effect of *Breynia rhamnoides* leaves extracts on Insulin Resistance on high fructose diet Induced C57BL/6J ob/ob diabetic mice blood serum after 21 days.

After 21 days study the plasma insulin resistance is increased up to 18.55 (pmol/l), the plasma insulin resistance decreases significantly ($p<0.01$) in all test drugs shown in table-6.

Effect of *Breynia rhamnoides* leaves extracts on triglycerides level on high fructose diet Induced C57BL/6J ob/ob diabetic mice blood serum after 21 days.

Significant ($p<0.01$) decreases triglycerides was observed in *Breynia rhamnoides* chloroform & methanolic leaves extracts and glibenclamide treatment groups when compared to control group.

Effect of *Breynia rhamnoides* leaves extracts on LDL level on high fructose Induced C57BL/6J ob/ob diabetic mice blood serum after 21 days.

The decreases **LDL** was observed in ***XDL* chloroform & methanolic leaves extracts** and glibenclamide treatment groups when compared to control group significantly ($p<0.01$).

Effect of *Breynia rhamnoides* leaves extracts on total cholesterol level on high fructose Induced C57BL/6J ob/ob diabetic mice blood serum after 21 days.

The decreases total cholesterol was observed in *Breynia rhamnoides* chloroform & methanolic leaves extracts and glibenclamide treatment groups when compared to control group Significant($p<0.01$).

The C57BL/6J ob/ob diabetic mice protective effect of beta cells of islet of langerhans on *fallowing drugs* leaves extract against high fructose induced diabetic C57BL/6J ob/ob diabetic mice.

After 21 days study the beta cells of islet of langerhans is increased up to 18.9±6.3 to 38.2±5.8 compared to the control group vs. test group significantly ($p<0.01$) which is shown in table-6.

Body Weight Changes

There was significant difference in mean body weight values between the fructose-fed control and *Breynia rhamnoides* leaves extract treated groups (250 ± 10 g, 248 ± 7 g and 212 ± 8 g, respectively) during the 21 days of the experimental period in the diabetic *ob/ob* mice model.

Histopathological Evaluation of Pancreas

The pancreas was isolated immediately after sacrificing the animal and washed with ice cold saline. It was then fixed in 10% neutral buffered formalin solution. Sections of 3-5µm thickness were stained with hematoxylin and eosin (H.E.) for histopathological examination. Diabetic C57BL/6J ob/ob diabetic mice revealed degenerative and lytic changes in islets of langerhans of pancreas similar to earlier study [31].

Table-1: Effect of *Breynia rhamnoides* methanolic extract (BRRME) on serum glucose (mg/dl) in high fructose diet induced diabetic c57bl/6j ob/ob diabetic mice.

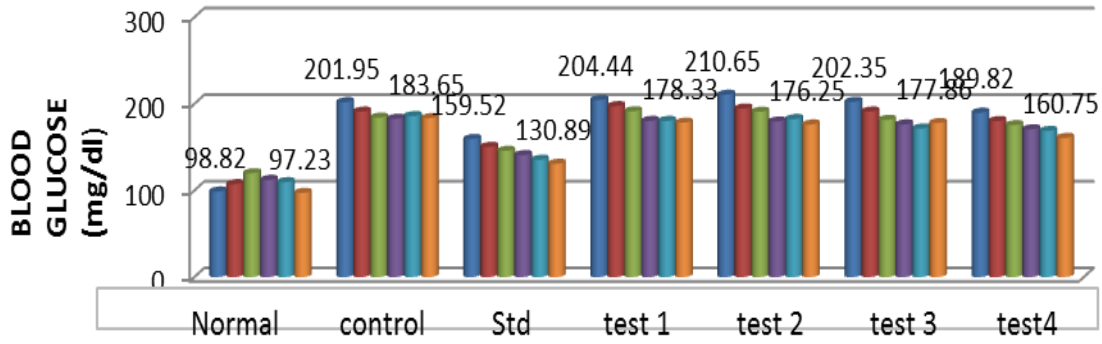
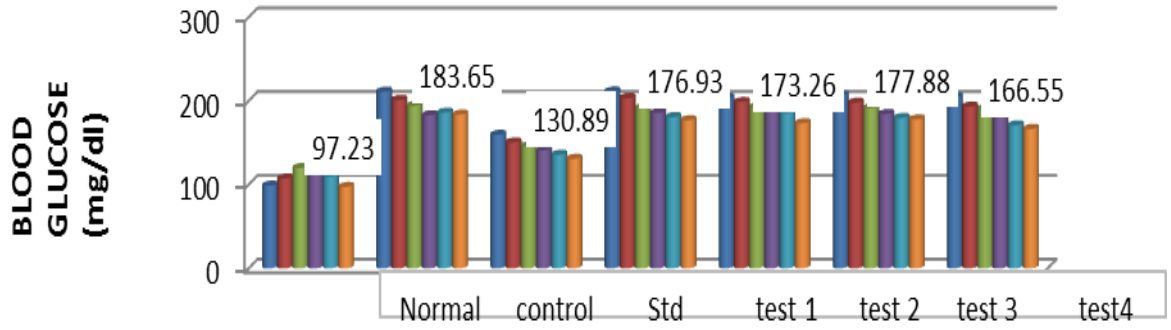
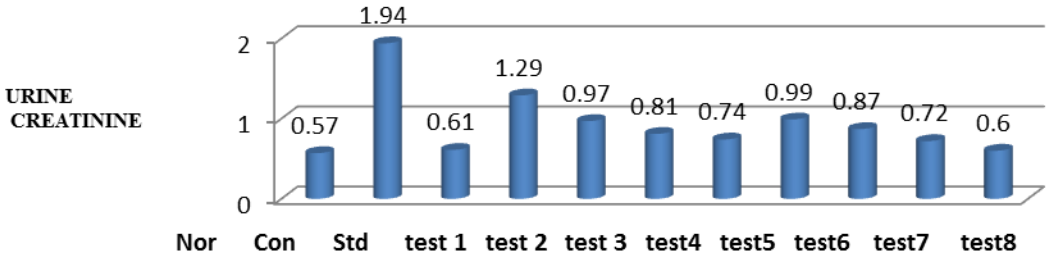


Table-2: Effect of *Breynia rhamnoides* chloroform leaves (BRRCE) extract on serum glucose (mg/dl) in high fructose diet induced diabetic c57bl/6j ob/ob diabetic mice.



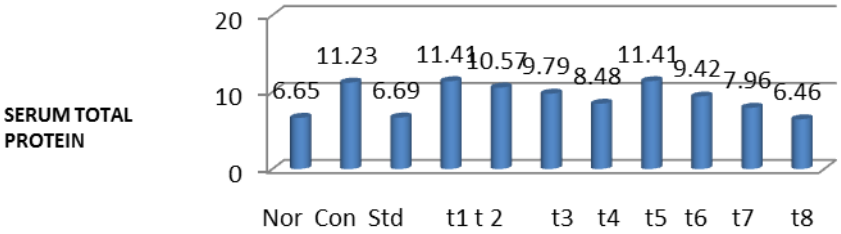
Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control.

Table-3: Effect of *Breynia rhamnoides* chloroform & methanolic leaves extracts on serum creatinine (mg/dl) in high fructose diet induced diabetic c57bl/6j ob/ob diabetic mice.



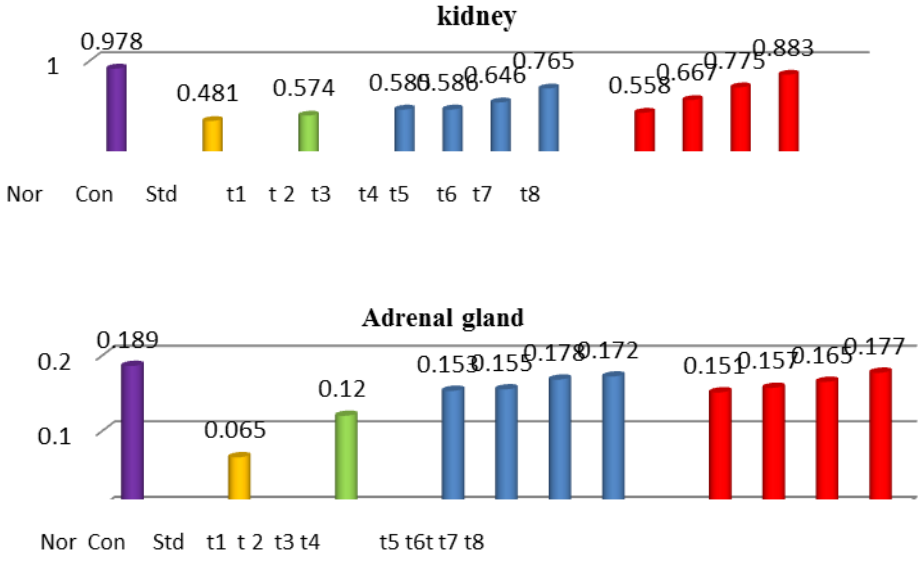
Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control.

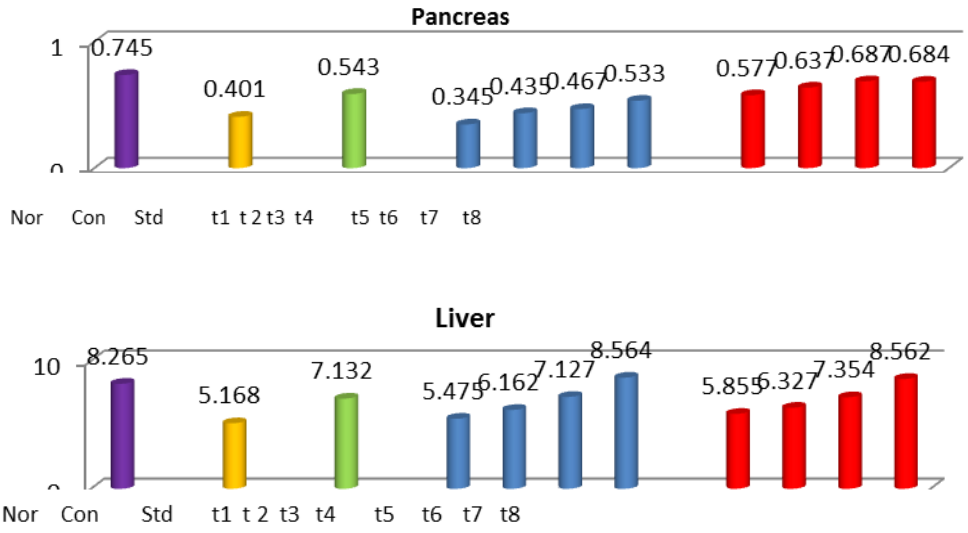
Table-4: Effect of *Breynia rhamnoides* chloroform & methanolic leaves extract on serum total protein (g/dl) in high fructose induced diabetic c57bl/6j ob/ob diabetic mice .



Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control.

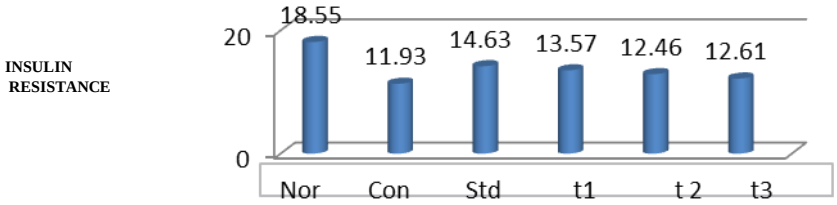
Table-5: Effect of *Breynia rhamnoides* chloroform & methanolic leaves extracts on high fructose diet organs to body weight ratio in c57bl/6j ob/ob diabetic mice.





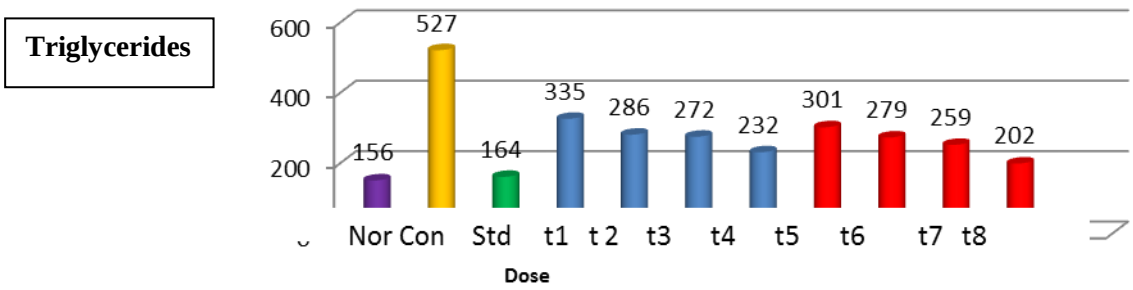
Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control.

Table-6: Effect of *Breynia rhamnoides* leaves extracts on insulin resistance on high fructose diet induced c57bl/6j ob/ob diabetic mice blood serum after 21 days.



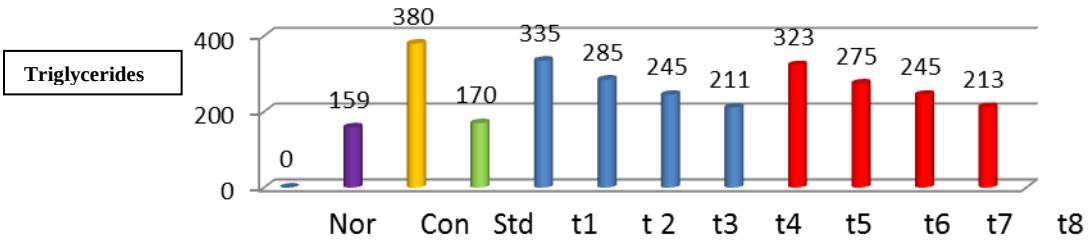
Fructose-induced insulin resistance: Evidence from euglycemic hyperinsulinemic clamp studies. Mean glucose levels (normal) were significantly higher in control vs. normal animals during the last 30 mins of the clamp period ($p < 0.01$).

Table-7: Effect of *Breynia rhamnoides* leaves extracts on triglycerides level on high fructose diet induced c57bl/6j ob/ob diabetic mice blood serum after 21 days.



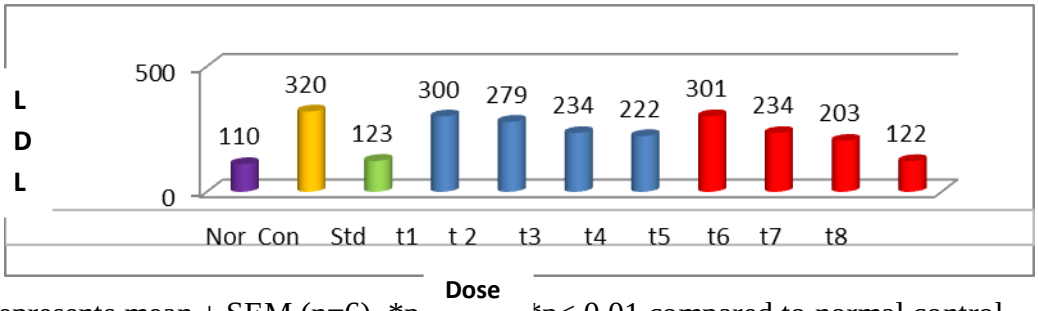
Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control.

Table-8: Effect of *Breynia rhamnoides* leaves extracts on total cholesterol level on high fructose induced c57bl/6j ob/ob diabetic mice blood serum after 21 days.



Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control.

Table-9: Effect of *Breynia rhamnoides* leaves extracts on ldl level on high fructose induced c57bl/6j ob/ob diabetic mice blood serum after 21 days.



Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control.

The protective effect of beta cells of islet of langerhans on *fallowing drugs* leaves extract against high fructose induced diabetic c57bl/6j ob/ob diabetic mice.

Name of drug	**Azimatetracantha leaves extract**
Treatment and dose	**Beta cells (per islet section**
Normal	46.8±5.6
Control	18.9±6.8
Chloroform extract(40 mg/kg)	25.7±5.7
Chloroform extract (80 mg/kg)	25.3±5.8
Chloroform extract (160 mg/kg)	24.3±3.7
Chloroform extract (200 mg/kg)	26.1±3.7*
Alcohol extract(40 mg/kg)	19.8±3.6
Alcohol extract(80 mg/kg)	22.3±9.7
Alcohol extract(160 mg/kg)	32.4±5.8**
Alcohol extract(200 mg/kg)	38.2±5.6**

Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control.

Histopathological Study

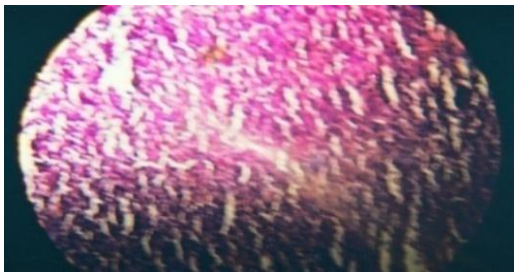


Fig-1 Effect of tween 80% on normal non diabetic C57BL/6J ob/ob diabetic

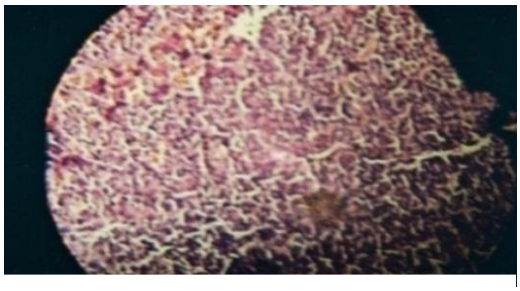


Fig-2 Effect on High Fructose Diet Induced Diabetic C57BL/6J Ob/Ob diabetic mice.

Fig-3 Effect of Glibenclamide (5 mg/kg) on high fructose induced diabetic C57BL/6J ob/ob diabetic mice.

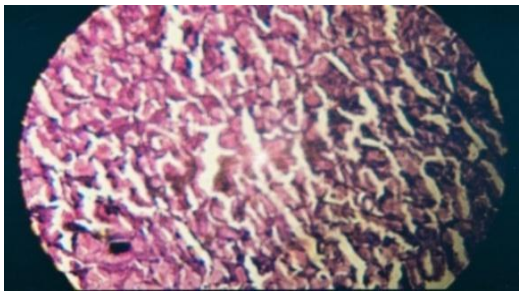


Fig-4 Effect of *Breynia rhamnoides* extract (40mg/kg) on high fructose induced diabetic C57BL/6J ob/ob diabetic mice pancreas.

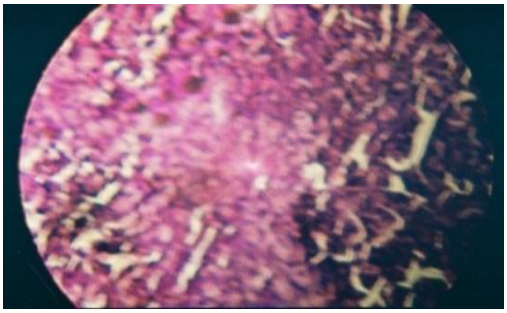


Fig-5 Effect of *Breynia rhamnoides* chloroform extract (80mg/kg) on high fructose induced diabetic C57BL/6J ob/ob diabetic mice pancreas.

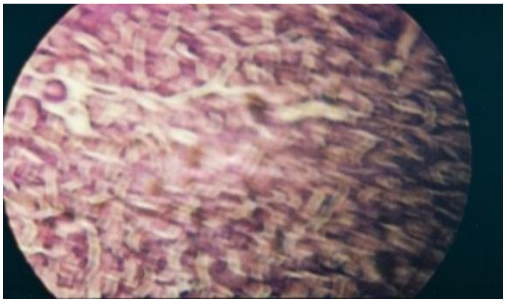


Fig-6 Effect of *Breynia rhamnoides* chloroform extract (160 mg/kg) on high fructose induced diabetic C57BL/6J ob/ob diabetic mice pancreas.

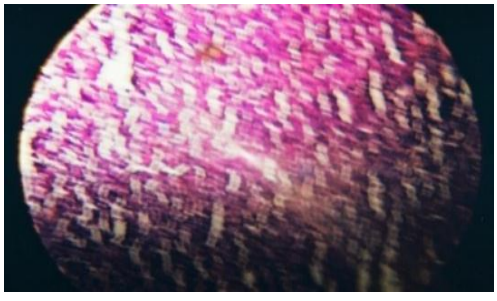


Fig-7 Effect of *Breynia rhamnoides* chloroform extract (200 mg/kg) on high fructose induced diabetic C57BL/6J ob/ob diabetic mice..

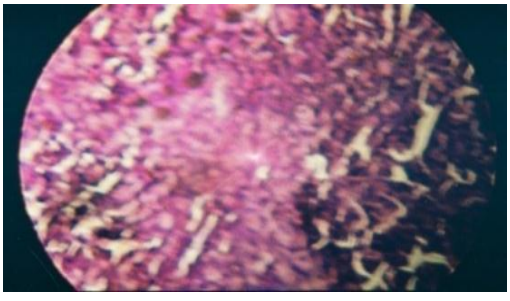


Fig-8 Effect of *Breynia rhamnoides* methanolic extract (40 mg/kg) on high fructose induced diabetic

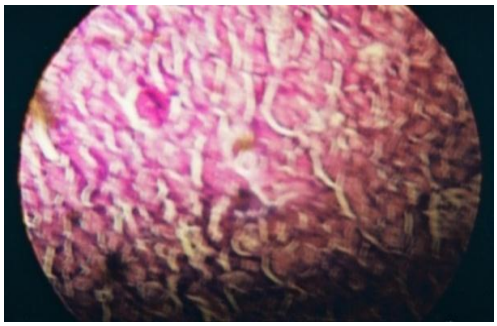


Fig-9 Effect of *Breynia rhamnoides* methanolic extract (80 mg/kg) on high fructose induced diabetic C57BL/6J ob/ob diabetic mice pancreas.

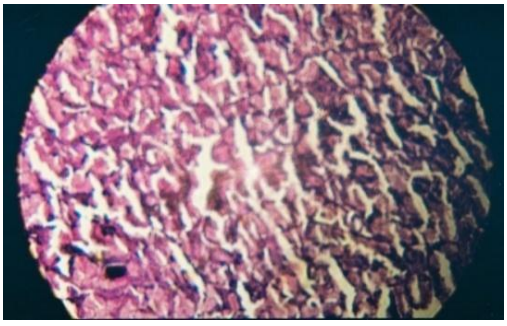


Fig-10 Effect of *Breynia rhamnoides* methanolic extract (160 mg/kg) on high fructose induced diabetic C57BL/6J ob/ob diabetic mice pancreas.

Fig-1. Section of pancreas showing normal architecture fig-2 showing inflammatory cellular infliction. Fig-3 showing enlarges pancreatic islets of langerhans.fig-5 showing slightly less severe fibrosis, inflammatory cellular infliction fig-6 showing enlarged and inflammatory cellular infliction edema fig-7 shows normal architecture of pancreatic islets of langerhans fig-8 Interstitial edema, and fibrosis and slightly enlarge pancreatic islets of langerhans fig-9 interstitial edema, and fibrosis and slightly enlarge pancreatic islets of langerhans.fig-10 interstitial edema, and fibrosis and slightly enlarge pancreatic islets of langerhans.

DISCUSSION

A high fructose diet induces insulin resistance, alterations in lipid metabolism, and oxidative stress in rat tissues [14]. Fructose is readily absorbed and rapidly metabolized by human liver. The exposure of the liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and triglycerides accumulation; which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance. These negative effects of fructose are the reason that fructose metabolism has gained recent research attention [15, 16]. The long-term negative effects can include changes in digestion, absorption, plasma hormone levels, appetite, and hepatic metabolism, leading to the development of insulin resistance, diabetes, obesity, and inevitably cardiovascular disease [17]. Because of its lipogenic properties, excess fructose in the diet can cause glucose and fructose malabsorption, together with greater elevations in triglycerides and cholesterol compared to other carbohydrates[18]. Therefore, fructose can uncontrollably produce glucose, glycogen, lactate, and pyruvate, providing both the glycerol and acyl portions of acyl-glycerol molecules. These particular substrates, and the resultant excess energy flux due to unregulated fructose metabolism, will promote the overproduction of triglycerides [19, 20]. A high fructose diet can have

hypertriglyceridemic and pro-oxidant effects, and fructose-fed rats have shown less protection from lipid peroxidation [21]. Oxidative stress has often been implicated in the pathology of insulin resistance induced by fructose feeding, and lipid peroxides, and reactive substances are undeniably elevated in fructose-fed animals, especially accompanying a deficient antioxidant system [22]. Administration of Breynia rhamnoides laves extract has been shown to prevent these changes, and improve insulin sensitivity[23] .In our study, treatment with Breynia rhamnoides also prevented several deleterious effects of fructose feed; such as the increases in serum glucose, cholesterol and triglyceride levels. In the other model the antihyperglycemic effects of Breynia rhamnoides leaves extract were evaluated in diabetic C57BL/6J ob/ob mice. Diabetic and obesity are complex genetic diseases caused by a combination of genetic predisposition and environmental exposure [24, 25]. The genetic contribution can be either monogenic or polygenic, with polygenic inheritance being the predominant mode of inheritance in human type- 2diabetes and obesity. The leptin mutations arose spontaneously in the C57BL/6J mice, and resulted in the severe early-onset of obesity, hyperphagia, hyperinsulinemia, and insulin resistance with modest hyperglycemia [14]. Leptin deficiency is associated with dyslipidemia (abnormal levels of cholesterol and triglycerides in the blood) and insulin resistance, a precursor to diabetes in animals and humans withlipoatrophy (fat loss) [27]. *Breynia rhamnoides* has been documented as a traditional treatment for diabetes. In the present study, chloroformic extract of *Breynia rhamnoides* and methanolic extract of *Breynia rhamnoides* (40, 80, 160 and 200 mg/kg) significantly decreased the serum glucose, triglycerides and cholesterol in the high fructose-induced C57BL/6J ob/ob mice. It also rendered nephroprotection by decreasing the total protein levels in urine. The alcoholic extract of *Breynia rhamnoides* has been shown to have a potential antioxidant activity in both in vitro and in vivo models [12]. However there is no evidence of any scientific studies into its antidiabetic activity.

SUMMARY AND CONCLUSION

In summary, our results on high fructose diet diabetic C57BL/6J ob/ob diabetic mice s that *Breynia rhamnoides* possess significant anti-hyperglycemic activity on chronic treatment, indicating its possible applications in type-2. More prominent and significant results were obtained with methanolic extract then compared to chloroform extract. However, both these extracts have failed to produce acute anti-hyperglycemic and hyperglycemic effects in all these models shows that, it has long-term anti-diabetic activity without hypoglycemic side effects. The anti-hyperglycemic effects observed with alcoholic extract were also equal to

Glibenclamide C57BL/6J ob/ob diabetic mice, it has potent and safe anti-diabetic component then compared to glibenclamide even in crude form. The histopathological study has shown anti-inflammatory, cellular infiltration, reduced interstitial edema, and fibrosis, it shows cytoprotective, and anti-lipedmic properties. The organ to body weight C57BL/6J ob/ob diabetic mice study indicated pancreatic and liver specific actions, thereby probably stimulating insulin secretion and other liver mediated hypoglycemic effects.

In conclusion *Breynia rhamnoides* alcoholic extract possess anti-diabetic, nephroprotective, cardioprotective and cytoprotective activities probably due to the presence of anti-oxidant (sterols)active constituents taraxerol and taraxerone present in high numbers in methanolic extract than compared to chloroform extract.

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