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EFFECT OF *XYLIA DOLABRIFORMIS* LEAVES EXTRACT ON HIGH FRUCTOSE DIET INDUCED C57BL/6J OB/OB DIABETIC MICE

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Keywords:

Xylia dolabriformis, High fructose diet, Anti-hyperglycemic, C57BL/6J*ob/ob* mice

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ABSTRACT: The objective of the present study was to investigate anti-diabetic and nephroprotective and cardioprotective activity of *Xylia dolabriformis* leaves extract, using High Fructose induced diabetic C57BL/6J*ob/ob* mice as model for clinical type-2 diabetic. At a regular interval of an 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 *Xylia dolabriformis* leaves at 40, 80, 160 and 200 mg/kg doses. *Xylia dolabriformis* 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 *Xylia dolabriformis* leaves. These results were also supported by histo-pathological studies. We conclude from the present study that *Xylia dolabriformis* alcoholic extract and chloroform extract long-term treatment may be beneficial in the management of diabetes.

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.

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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].

Development of safe antidiabetic agents is still a challenge for scientists working in this area. 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 s (涤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 antidiabetic drugs. This study was designed to examine the possibility of the antidiabetic effect of *Xylia dolabriformis* leaves (XDL) (Leguminosae) 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 *Xylia dolabriformis* 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 *Xylia dolabriformis* 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 *Xylia dolabriformis* leaves extract in type 2 diabetes or an insulin resistant animal model.

Based on these profiles of *Xylia dolabriformis* leaves, work was undertaken to screen the leaves of the plant for its antidiabetic activity in type 2 diabetes.

MATERIAL AND METHODS:

PrepaC57BL/6J ob/ob diabetic mice ion of plant extract:

Plant material collection and authentication: In the present study, the *Xylia dolabriformis* 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 *Xylia dolabriformis* 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 C57BL/6J ob/ob diabetic mice ories 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, India.

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 (20-22ºC 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 *Xylia dolabriformis* chloroformic and methanolic leaves extract 40, 80, 160 and 200mg/kg bw po.

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.

Estimation of Serum and Urine Bio-Chemical Parameters:

1. **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.

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

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

4. **Estimation of HDL:** The blood HDL level will be estimated by Cholesterol and HDL Cholesterol Kit (MR) by auto- analyzer**.**

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

6. **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.

7. **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:

Body weight changes: There was significant difference in mean body weight values between the fructose-fed control and *Xylia dolabriformis* 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.

Effect of *Xylia dolabriformis* 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 [**Graph 1**] and 183.65 mg/dl in chloroformic extract [**Graph 2**]. Administration of C57BL/6J ob/ob diabetic mice chloroform and methanolic extract of *Xylia dolabriformis* 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 (Graph 1).

Effect of *Xylia dolabriformis* 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 higher ($P<0.001$, 532.83 g/dL, $P<0.05$) in the diabetic control group than in the normal animals. In the *Xylia dolabriformis* leaves extract administered group of animals the triglyceride (380.33 g/dL, $P<0.001$) and cholesterol levels (211 g/dL, $P<0.05$) were significantly lower than in the diabetic control group of animals (Graph 1).

Effect of *Xylia dolabriformis* leaves extract in high fructose diet-induced change in serum total protein biochemical parameter: The total protein in the urine of the *Xylia dolabriformis* leaves extract (40, 80, 160, 200 mg/kg)-treated group of animals reduced significantly (11.23 to 6.46 g/dL, $P < 0.05$) (**Graph 4**), suggesting partly the nephron-protective activity of the drug. Low dose of *Xylia dolabriformis* leaves extract (40 mg/kg) was not significant in showing nephroprotective activity. However the nephroprotective effect of *Xylia dolabriformis* leaves extract needs further study.

The results of our study indicate that the development of hyperglycemia and progression of diabetes by feeding a high fructose diet and *Xylia dolabriformis* leaves extract might be prevented and/or delayed.

Effect of *Xylia dolabriformis* 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 *Xylia dolabriformis* 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. Glibenclamide and all treatment group treatment for 21 day's concomitant with high fructose diet feeding, significantly ($p<0.001$) reduced loss of total proteins induced by high fructose diet significantly elevated loss of urinary total proteins was noted in all treatment groups with all doses selected in the present study.

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

Effect of *Xylia dolabriformis* 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 *Azima tetracantha* chloroform & methanolic leaves extracts and glibenclamide treatment groups when compared to control group.

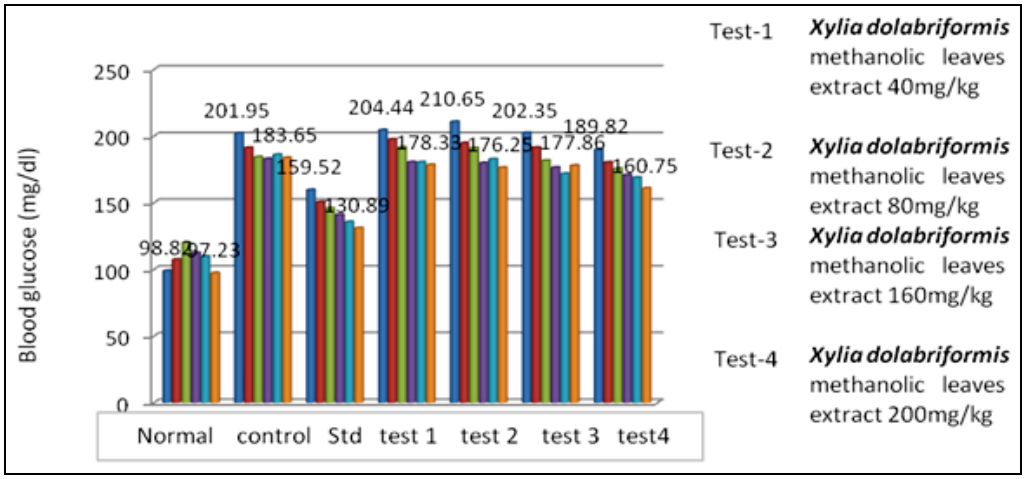
Effect of *Xylia dolabriformis* leaves extracts on LDL level on high fructose Induced C57BL/6J ob/ob diabetic mice blood serum after 21 days: The decreases LDL was observed in XDL chloroform & methanolic leaves extracts and glibenclamide treatment groups when compared to control group significantly ($p<0.01$).

Effect of *Xylia dolabriformis* 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 *Xylia dolabriformis* chloroform & methanolic leaves extracts and glibenclamide treatment groups when compared to control group Significant($p<0.01$).

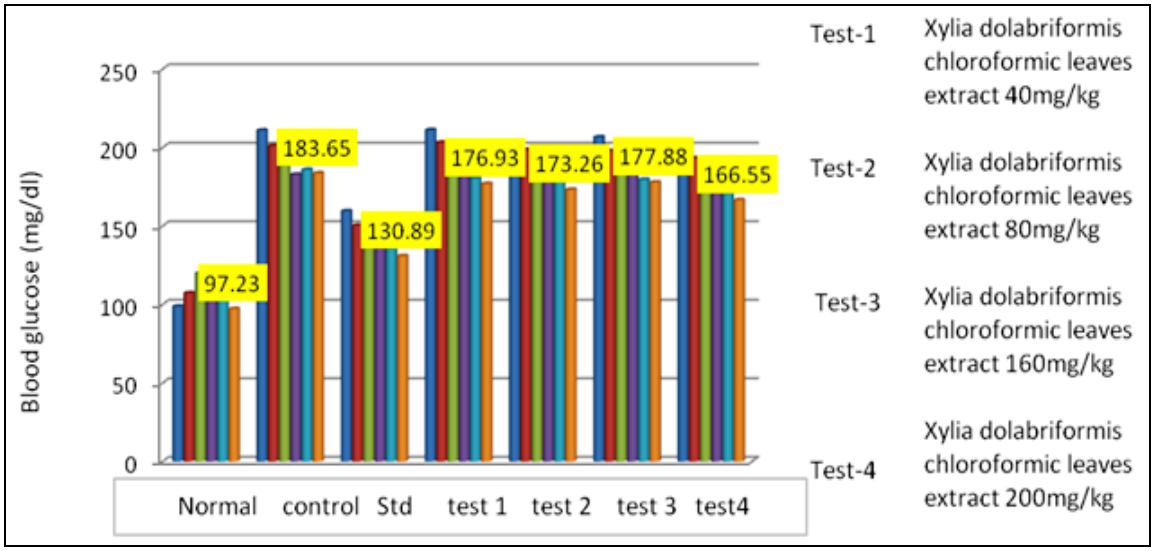
The C57BL/6J ob/ob diabetic mice protective effect of beta cells of Islet of Langerhans on *fallowing drugs* leaves extract against high fructose induced diabetic C57BL/6J ob/ob diabetic mice: After 21 days study the beta cells of Islet of Langerhans is increased up to 18.9±6.3 to 38.2±5.8 compared to the control group vs. test group significantly ($p<0.01$) which is shown in **Graph** 6.

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]. It was also observed the islets were shrinken, inflammatory cellular infliction with fibrosis.

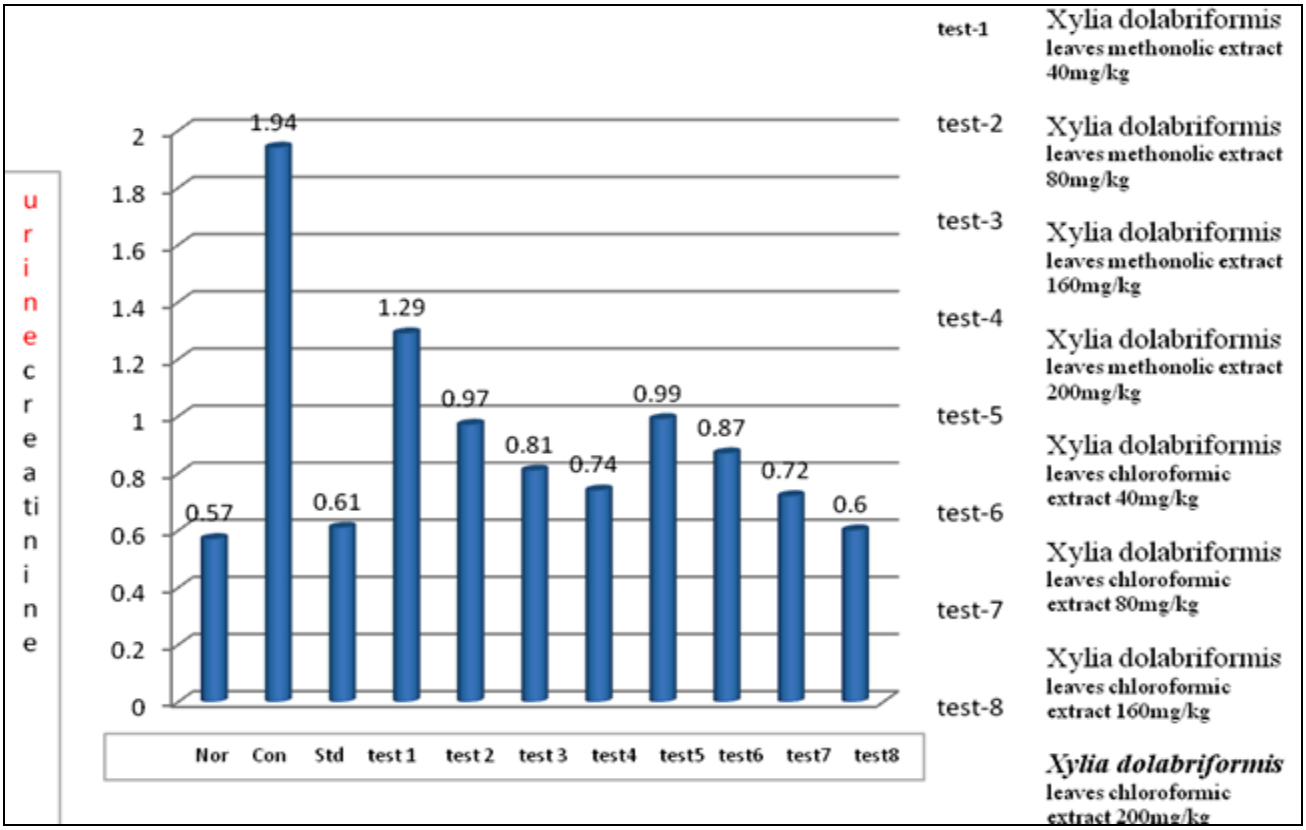
Treatment of diabetic C57BL/6J ob/ob diabetic mice with glibenclamide inhibited high fructose diet induced shrinking of Islets of Langerhans of the pancreas, inflammatory cellular infliction and enlarged pancreatic cells. *Xylia dolabriformis* leaves methanolic and chloroform extract treatment reversed all the effects of high fructose diet linear and dose dependently. However, more prominent effect of methanolic extract was observed than the chloroform extract. Higher dose increased the size of Islets of Langerhans inhibited lymphocytic infliction and vascular changes. The effect of methanolic extract was much higher than the chloroform extract and shows inhibitory effect only on lysis and shrinking (**figures 1 - 10**).



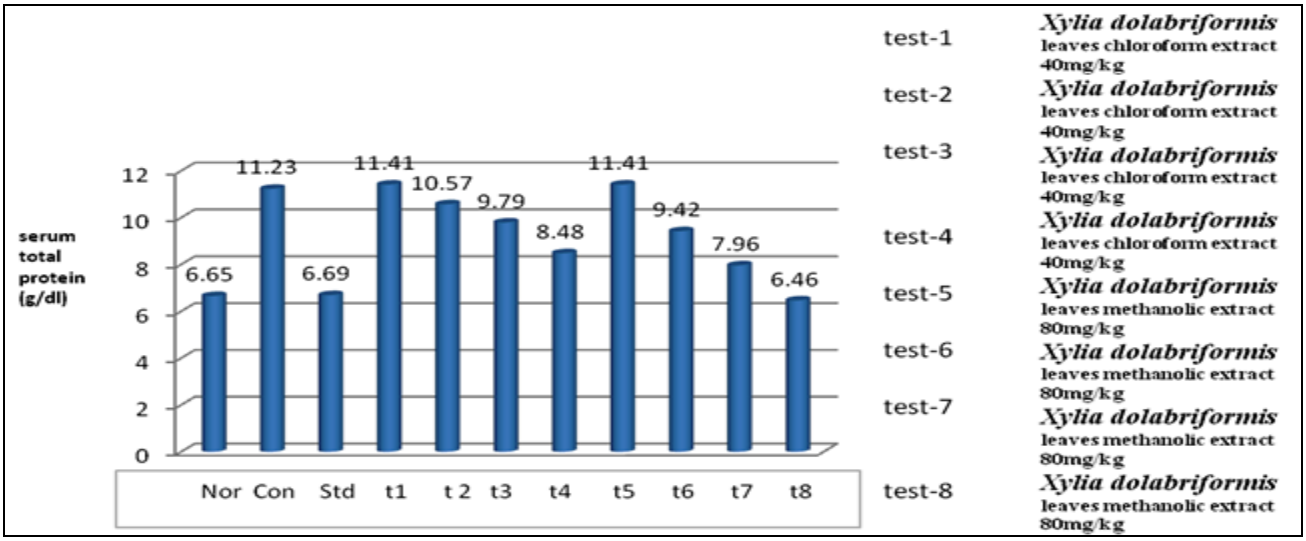
GRAPH 1: EFFECT OF *XYLIA DOLABRIFORMIS* METHANOLIC LEAVES EXTRACT ON SERUM GLUCOSE (mg/dl) IN HIGH FRUCTOSE DIET INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE



GRAPH 2: EFFECT OF *XYLIA DOLABRIFORMIS* CHLOROFORM LEAVES EXTRACT ON SERUM GLUCOSE (mg/dl) IN HIGH FRUCTOSE DIET INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE. Effect of normal (saline), tween 80 (2%), Glibenclamide (Control; 5 mg/kg), XDL chloroform extract 40, 80,160 and 200 mg/kg and alcoholic extract 40, 80, 160 and 200 mg/kg on serum glucose in high-fructose diet induced diabetic C57BL/6J ob/ob diabetic mice. Retro-orbital blood was collected, centrifuged and serum was sepaC57BL/6J ob/ob diabetic mice ed. Serum glucose level was estimated by using commercially available glucose kit on 1st, 7th, 14th, and 21st days. The results were analyzed by student't' test and by comparing mean of treatment group with control and control group with normal for coming to conclusion and presented as Mean± S.E.M. The 'p' value <0.05 was considered as significant. *p<0.05, **p<0.01, ***p<0.001.

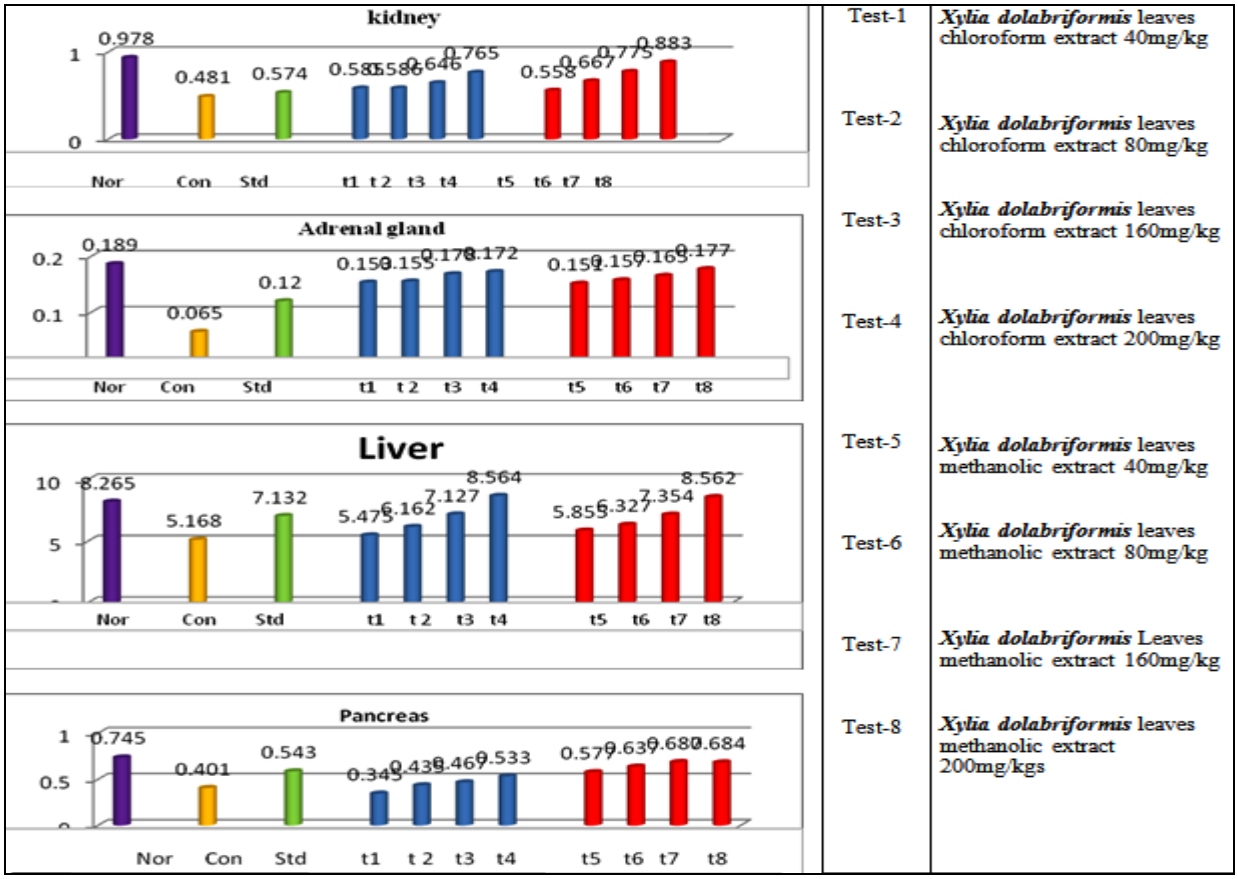


GRAPH 3: EFFECT OF *XYLIA DOLABRIFORMIS* CHLOROFORM & METHANOLIC LEAVES EXTRACTS ON SERUM CREATININE (mg/dl) IN HIGH FRUCTOSE DIET INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE. Effect of normal (saline), tween 80 (2%), glibenclamide (5 mg/kg), of *XDL* chloroform & methanolic leaves extracts 40, 80,160 and 200 mg/kg on urine creatinine in high fructose diet induced diabetic C57BL/6J ob/ob diabetic mice. Urine was collected on 16th day; creatinine were estimated by using creatinine kit by auto analyzer. The results were analyzed by student't' test and by comparing mean of treatment group with control and control group with normal for coming to conclusion and presented as mean± SEM. The 'p' value <0.05 was considered as significant. *p<0.05, **p<0.01, ***p<0.001.



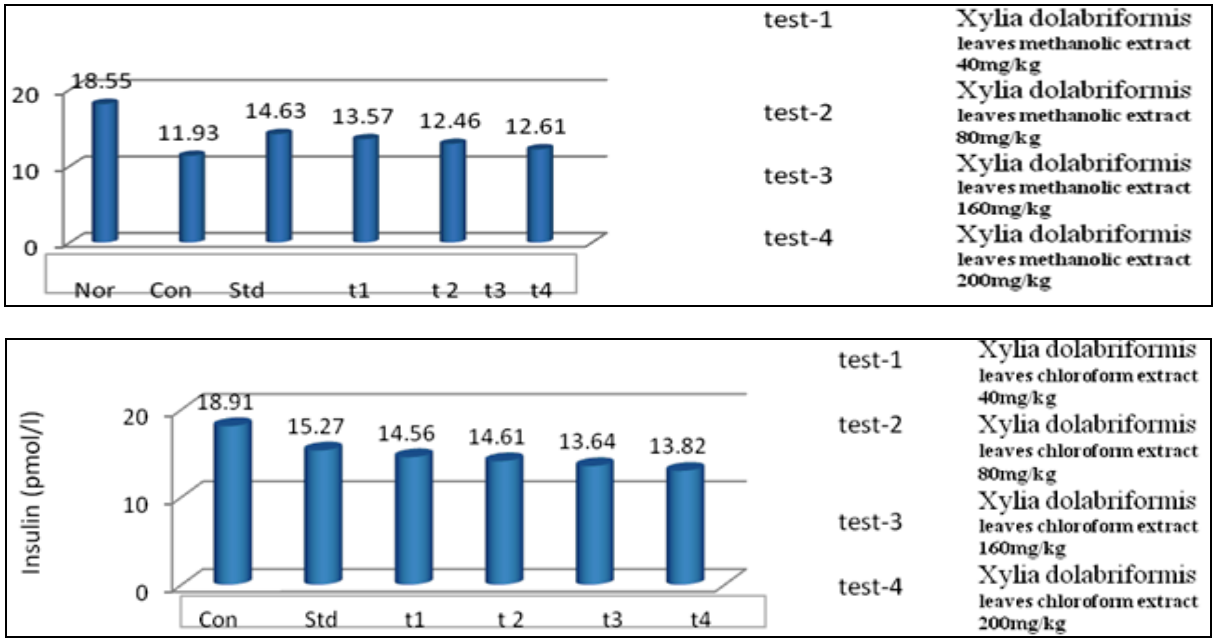
GRAPH 4: EFFECT OF *XYLIA DOLABRIFORMIS* CHLOROFORM & METHANOLIC LEAVES EXTRACT ON SERUM TOTAL PROTEIN (g/dl) IN HIGH FRUCTOSE INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE

Effect of normal (saline), tween 80 (2%), glibenclamide (5 mg/kg) *XDL* chloroform & methanolic leaves extract 40, 80,160 and 200 mg/kg on total protein in high fructose diet induced diabetic C57BL/6J ob/ob diabetic mice. Serum was collected on 16th day; total protein were estimated by using Biuret method by semi auto analyzer. The results were analyzed by student 't' test and by comparing mean of treatment group with control and control group with normal for coming to conclusion and presented as mean± SEM. The 'p' value <0.05 was considered as significant. *p<0.05, **p<0.01, ***p<0.001.

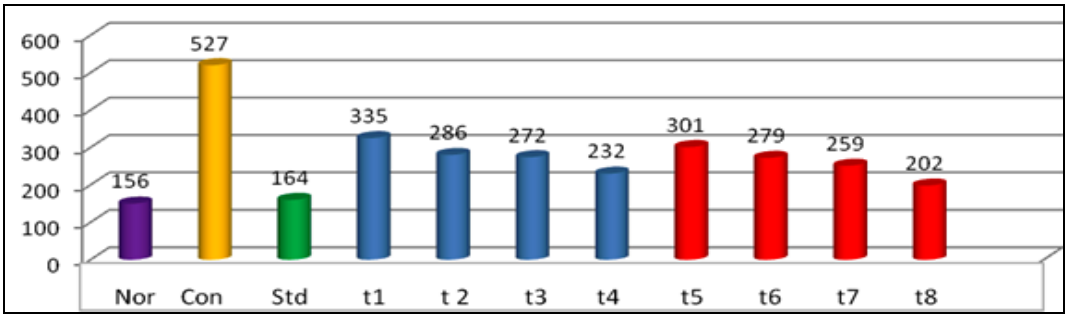


GRAPH 5: EFFECT OF *XYLIA DOLABRIFORMIS* CHLOROFORM & METHANOLIC LEAVES EXTRACTS ON HIGH FRUCTOSE DIET ORGANS TO BODY WEIGHT RATIO IN C57BL/6J OB/OB DIABETIC MICE.

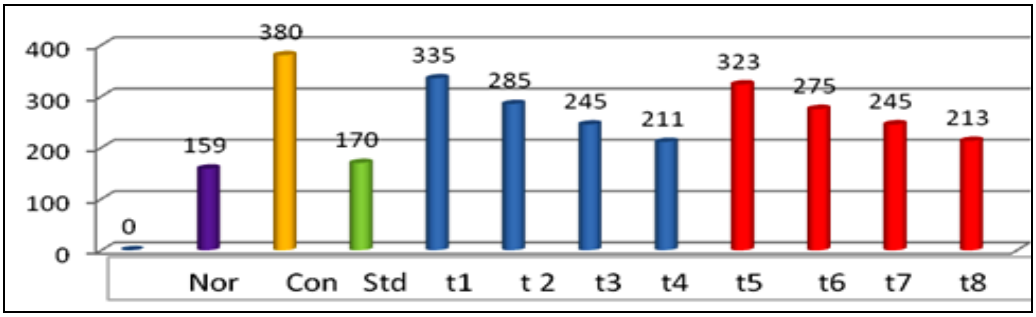
Effect of normal (saline), Glibenclamide (5 mg/kg), chloroform extract 40, 80,160 and 200 mg/kg and alcoholic extract 40, 80, 160 and 200 mg/kg on organs to body weight C57BL/6J ob/ob diabetic mice io in (mg/gm) STZ -induced diabetic mice. On the final day adrenal glands, kidney, liver, heart and pancreas were isolated and weighed in wet condition to measure organ to body weight C57BL/6J ob/ob diabetic mice io. The results were analyzed by student't' test and by comparing mean of treatment group with control and control group with normal for coming to conclusion and presented as Mean± S.E.M. The 'p' value <0.05 was considered as significant. *p<0.05, **p<0.01, ***p<0.001.



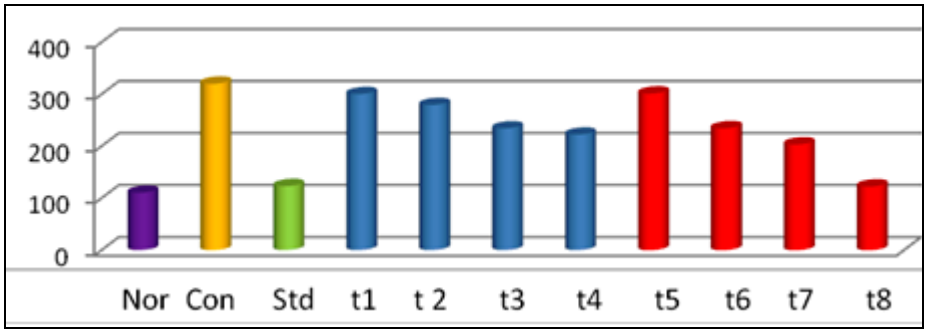
GRAPH 6: EFFECT OF *XYLIA DOLABRIFORMIS* LEAVES EXTRACTS ON INSULIN RESISTANCE ON HIGH FRUCTOSE DIET INDUCED C57BL/6J OB/OB DIABETIC MICE BLOOD SERUM AFTER 21 DAYS. Fructose-induced insulin resistance: Evidence from Euglycemic hyperinsulinemic clamp studies. Mean glucose levels (normal) were significantly higher in control vs. normal animals during the last 30 mins of the clamp period ($p < 0.01$). Mean insulin levels (test-1) were significantly higher in the control vs. test-1 hamsters during the clamp period.



GRAPH 7: EFFECT OF *XYLIA DOLABRIFORMIS* LEAVES EXTRACTS ON TRIGLYCERIDES LEVEL ON HIGH FRUCTOSE DIET INDUCED C57BL/6J OB/OB DIABETIC MICE BLOOD SERUM AFTER 21 DAYS. Effect of normal (saline), tween 80 (2%), glibenclamide (5 mg/kg) *XDL* chloroform & methanolic leaves extract 40, 80,160 and 200 mg/kg on total protein in high fructose diet induced diabetic C57BL/6J ob/ob diabetic mice. Serum was collected on 16th day; triglycerides were estimated by using GPO- Triender method, by semi auto analyzer. The results were analyzed by student 't' test and by comparing mean of treatment group with control and control group with normal for coming to conclusion and presented as mean± SEM. The 'p' value <0.05 was considered as significant. *p<0.05, **p<0.01, ***p<0.001.



GRAPH 8: EFFECT OF *XYLIA DOLABRIFORMIS* LEAVES EXTRACTS ON TOTAL CHOLESTEROL LEVEL ON HIGH FRUCTOSE INDUCED C57BL/6J OB/OB DIABETIC MICE BLOOD SERUM AFTER 21 DAYS. Effect of normal (saline), tween 80 (2%), glibenclamide (5 mg/kg) *XDL* chloroform & methanolic leaves extract 40, 80,160 and 200 mg/kg on total protein in in high fructose diet induced diabetic C57BL/6J ob/ob diabetic mice. Serum was collected on 16th day; triglycerides were estimated by using Cholesterol Kit (MR), by semi auto analyzer. The results were analyzed by student 't' test and by comparing mean of treatment group with control and control group with normal for coming to conclusion and presented as mean± SEM. The 'p' value <0.05 was considered as significant. *p<0.05, **p<0.01, ***p<0.001.



GRAPH 9: EFFECT OF *XYLIA DOLABRIFORMIS* LEAVES EXTRACTS ON LDL LEVEL ON HIGH FRUCTOSE INDUCED C57BL/6J OB/OB DIABETIC MICE BLOOD SERUM AFTER 21 DAYS. Effect of normal (saline), tween 80 (2%), glibenclamide (5 mg/kg) *XDL* chloroform & methanolic leaves extract 40, 80,160 and 200 mg/kg on total protein in in high fructose diet induced diabetic C57BL/6J ob/ob diabetic mice. Serum was collected on 16th day; triglycerides were estimated by using GPO Method by using semi auto analyzer. The results were analyzed by student 't' test and by comparing mean of treatment group with control and control group with normal for coming to conclusion and presented as mean± SEM. The 'p' value <0.05 was considered as significant. *p<0.05, **p<0.01, ***p<0.001.

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	*Azima tetracantha* 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**

Effect of normal (saline), tween 80 (2%), glibenclamide (5 mg/kg) *Azima tetracantha* chloroform & methanolic leaves extract 40, 80,160 and 200 mg/kg on total beta cell **count in high fructose diet** induced diabetic C57BL/6J ob/ob diabetic mice. On the final day pancreas were isolated and beta-cell were counted by using neumberes chamber by using compound microscope. The results were analyzed by student't' test and by comparing mean of treatment group with control and control group with normal for coming to conclusion and presented as Mean± S.E.M. The 'p' value <0.05 was considered as significant. *p<0.05, **p<0.01, ***p<0.001.

Histopathological Study: Figures 1- 10.

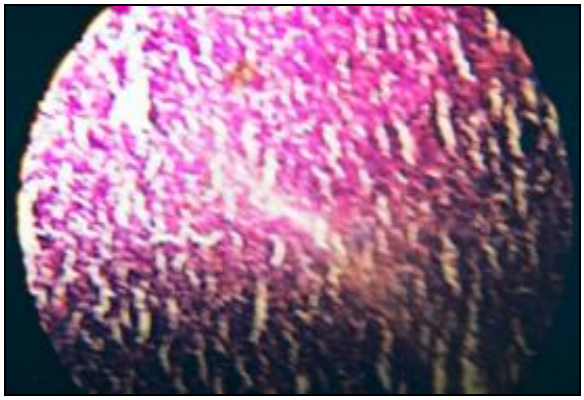


FIG. 1: EFFECT OF TWEEN 80% ON NORMAL NON DIABETIC C57BL/6J OB/OB DIABETIC MICE PANCREAS. Section of pancreas showing normal architecture of islets of Langerhans

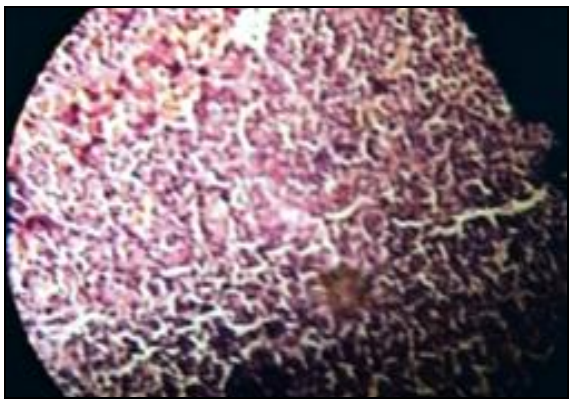


FIG. 2: EFFECT ON HIGH FRUCTOSE DIET INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE. Section of pancreas showing inflammatory cellular infiltC57BL/6J ob/ob diabetic mice ion, shrinkinen islets with lytic, vascular changers, interstitial edema, cellular necrosis and fibrosis

FIG. 3: EFFECT OF GLIBENCLAMIDE (5 mg/kg) ON HIGH FRUCTOSE INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE. Sections of pancreas showing enlarge pancreatic islets of Langerhans, inflammatory cellular infiltC57BL/6J ob/ob diabetic mice ion and cellular necrosis.

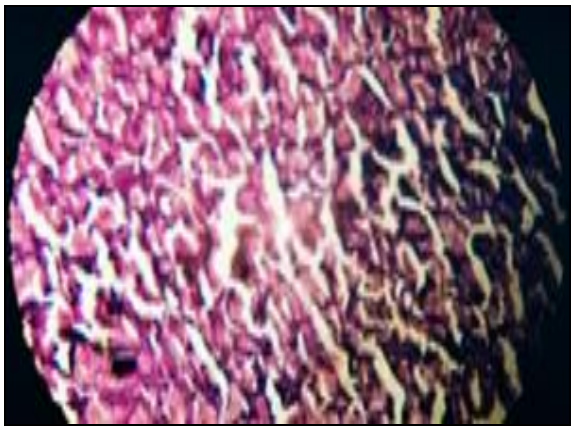


FIG. 4: EFFECT OF *XYLIA DOLABRIFORMIS* CHLOROFORM EXTRACT (40mg/kg) ON HIGH FRUCTOSE INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE PANCREAS. Sections of pancreas showing marked hyperplasia, inflammatory cellular infiltC57BL/6J ob/ob diabetic mice ion and fibrosis and shrinken pancreatic Islets of Langerhans.

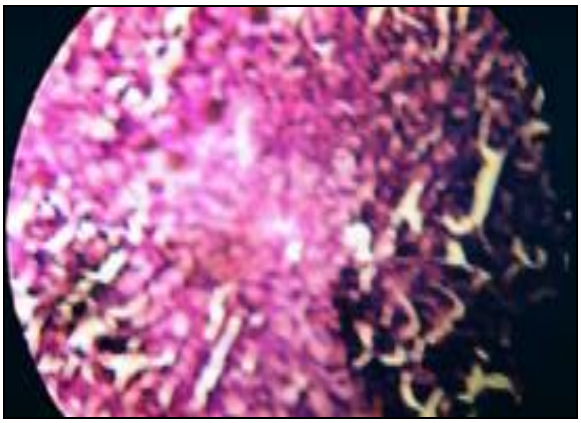


FIG. 5: EFFECT OF *XYLIA DOLABRIFORMIS* CHLOROFORM EXTRACT (80mg/kg) ON HIGH FRUCTOSE INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE PANCREAS. Section showing slightly less severe fibrosis, inflammatory cellular infiltC57BL/6J ob/ob diabetic mice ion, cellular necrosis and slightly enlarge pancreatic Islets of Langerhans.

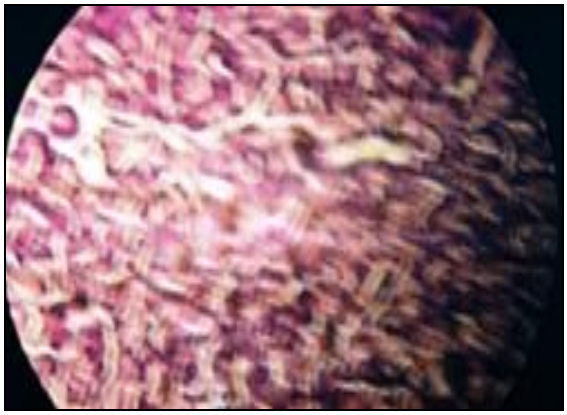


FIG. 6: EFFECT OF *XYLIA DOLABRIFORMIS* CHLOROFORM EXTRACT (160mg/kg) ON HIGH FRUCTOSE INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE PANCREAS. Section of pancreas showing enlarged pancreatic Islets of Langerhans, inflammatory cellular infiltC57BL/6J ob/ob diabetic mice ion and interstitial edema.

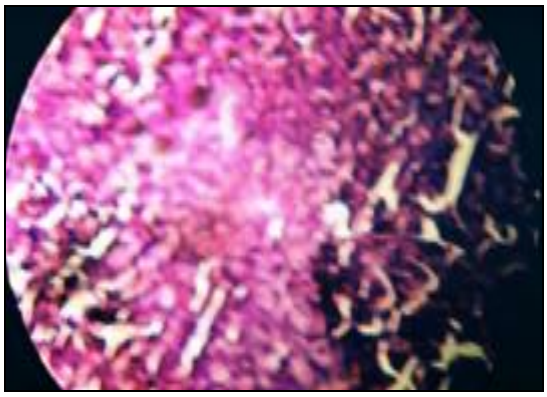


FIG. 7: EFFECT OF *XYLIA DOLABRIFORMIS* CHLOROFORM EXTRACT (200mg/kg) ON HIGH FRUCTOSE INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE PANCREAS. Section of pancreas showing normal architecture of pancreatic islets of Langerhans

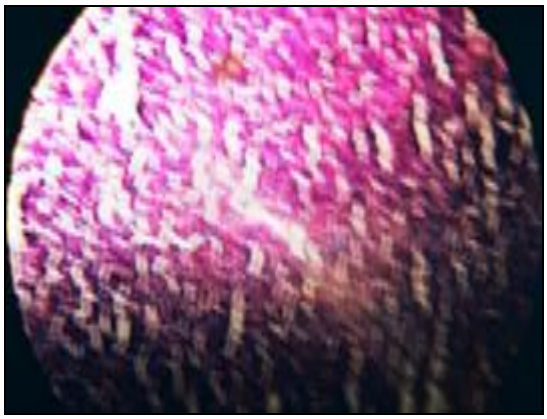


FIG. 8: EFFECT OF *XYLIA DOLABRIFORMIS* METHANOLIC EXTRACT (40mg/kg) ON HIGH FRUCTOSE INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE PANCREAS. Section of pancreas showing cellular necrosis, inflammatory cellular infiltration vascular changes and shrinken pancreatic islets of Langerhans.

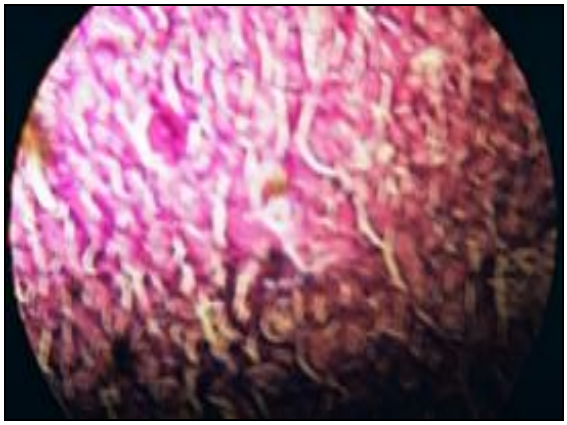


FIG. 9: EFFECT OF *XYLIA DOLABRIFORMIS* METHANOLIC EXTRACT (80mg/kg) ON HIGH FRUCTOSE INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE PANCREAS. Section of pancreas showing slight inflammatory cellular infiltC57BL/6J ob/ob diabetic mice ion, interstitial edema, and fibrosis and slightly enlarge pancreatic islets of Langerhans.

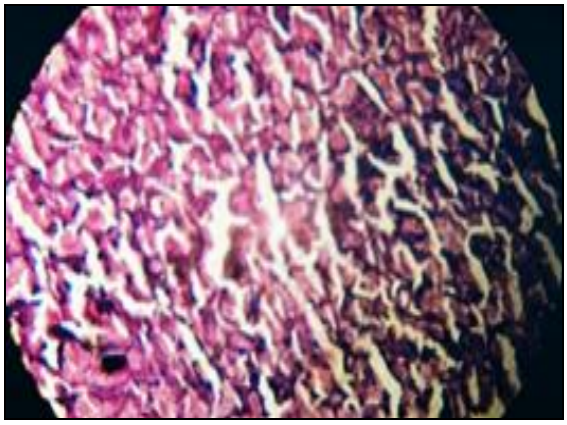


FIG. 10: EFFECT OF *XYLIA DOLABRIFORMIS* METHANOLIC EXTRACT (160mg/kg) ON HIGH FRUCTOSE INDUCED DIABETIC C57BL/6J OB/OB DIABETIC MICE PANCREAS. Section of pancreas showing slight inflammatory cellular infiltC57BL/6J ob/ob diabetic mice ion, interstitial edema, and fibrosis and slightly enlarge pancreatic 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. High fructose diet (85-100 g/per day) has resulted in significant increases insulin sensitivity.

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].

In the liver, fructose is metabolized into glyceraldehyde and dihydroxyacetone phosphate. These particular fructose end products can are readily taken up in the glycolytic pathway. The key importance is the ability of fructose to bypass the main regulatory step of glycolysis; the conversion of glucose-6-phosphate to fructose 1, 6-bisphosphate, controlled by phosphofructokinase. Thus, while glucose metabolism is negatively regulated by phosphofructokinase, fructose can continuously enter the glycolytic pathway.

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 *Xylia dolabriformis* leaves extract has been shown to prevent these changes, and improve insulin sensitivity [23]. In our study, treatment with *Xylia dolabriformis* 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 *Xylia dolabriformis* leaves extract were evaluated in diabetic C57BL/6J ob/ob mice.

Diabetes 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- 2 diabetes and obesity. The leptin mutations arose spontaneously in the C57BL/6J mice, and resulted in the severe early-onset of obesity, hyperphagia, hyperinsulin-emia, 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 with lipoatrophy (fat loss) [27].

Xylia dolabriformis has been documented as a traditional treatment for diabetes. In the present study, chloroformic extract of *Xylia dolabriformis* and methanolic extract of *Xylia dolabriformis* (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 *Xylia dolabriformis* 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. The chemical investigation of the leaves extract of *Xylia dolabriformis* revealed the presence of *taraxerol and taraxerone*. The presence of *taraxerol and taraxerone* supports its antioxidant potential. *taraxerol and taraxerone* has been shown to normalize blood sugar and insulin levels in type 2 diabetics[13]. The mechanism for this effect is that *taraxerol and taraxerone* stimulates the release of insulin in the presence of non-stimulatory glucose concen- trations and inhibits glucose-6-phosphatase [29].

Taraxerol and taraxerone reduces elevated blood glucose levels by the downregulation of glucose-6-phosphatase. Human liver microsome studies show that *taraxerol and taraxerone* inhibits cholesterol absorption. In a clinical study *taraxerol and taraxerone* was shown to lower cholesterol and triglyceride levels [13]. These results indicate a possible antihyperglycemic use for *taraxerol and taraxerone* in the prevention and treatment of diabetes. *Taraxerol and taraxerone has* ability to protect cells and tissues from oxidative stress, which induces the formation of cytoprotective enzymes like catalase and super- oxide dismutase [30]. Furthermore, the treatment of chloroform and methanolic extracts of XDL leave showed an improved lipid profile by reducing the level of total cholesterol and triglycerides.

Plant steroidal (*taraxerol and taraxerone*) found in the *Xylia dolabriformis* extract may have contributed to the improvement in lipid profile. This antihyperlipidemic effect could represent a protective mechanism against the development of hypercholestemia complications associated with diabetes. Chloroform extract of *Xylia dolabriformis* and methanolic extract of *Xylia dolabriformis* decreased the serum glucose, cholesterol and triglyceride levels of the diabetic C57BL/6J mice, suggesting an anti- hyperglycemic effect. The principal antioxidant compounds (*taraxerol and taraxerone*)) of *Xylia dolabriformis* extract may also be responsible for antibibetic activity.

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SUMMARY AND CONCLUSION: In summary, our results on high fructose diet diabetic C57BL/6J ob/ob diabetic mice s that *Xylia dolabriformis* 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 demonst 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 *Xylia dolabriformis* alcoholic extract possess anti-diabetic, nephroprotective, cardio-protective 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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