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PANCREATIC BETA CELL PROTECTIVE EFFECTS OF *FLEMENGIA STROBILIFERA* EXTRACTS IN COMBINATION MODEL OF HIGH FAT DIET AND STREPTOZOTOCIN INDUCED TYPE 2 DIABETES

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Keywords:

Diabetes, *Flemengia strobilifera*, High fat diet, Beta cells

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ABSTRACT: The current study deals with anti-diabetic and pancreatic protective effects of *Flemengia strobilifera* extracts in a high-fat diet (HFD) and streptozotocin (STZ) induced type 2 diabetes model. Toluene extracts were orally administered at the dose of 10, 30 and 100 mg/kg and metformin was used as a standard anti-diabetic drug at 10 mg/kg dose orally. Blood glucose, lipid profiles, pancreatic oxidative stress parameters and oral glucose tolerance test (OGTT) were performed. Treatment with extracts resulted in a significant decrease in diabetes incidence with a marked reduction in the blood glucose levels dose dependent and time dependent. Glucose tolerance was greatly improved after treatment with plant extracts with high dose group producing effects similar to standard drug-mediated beneficial effects. Histopathological studies also indicated pancreatic beta-cell protective effects of *Flemengia strobilifera* extracts with increased beta-cell number and size. Our results demonstrate that *Flemengia strobilifera* can be a possible remedy to treat type 2 diabetes and associated complications.

INTRODUCTION: The incidence of diabetes and its associated complications are posing serious health concern globally due to increased industrialization and increased cafeteria dietary habits [1]. The imbalance between energy intake and expenditure may result in excessive accumulation of lipids in various parts of body mainly skeletal muscle and liver. The sequence of events generally happens in diabetes are poor every expenditure, dyslipidemia, Insulin resistance metabolic syndrome, glucose intolerance, pre-diabetes, diabetes and finally diabetic complications [2, 3].

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The mainstay of therapy for diabetes is subcutaneous insulin which involves a lot of pain and scope of development of hypoglycemia upon in appropriate dosing [4, 5]. The existing oral hypoglycemic agents are effective only to control the blood glucose levels to only some extent and they also pose several side effects upon the long term. Despite great advancements in diabetes research, the quest for efficient and safer anti-diabetic drugs is still active [6]. There is no drug that can prevent or reverse diabetes.

In general natural products possess multiple pharmacological properties and considered relatively safer compared to synthetic drugs [7, 8]. Several natural products and plant extracts have been explored to treat diabetes and associated diabetic complications. In the current study, we have designed an experimental model to evaluate the protective effects of *Flemengia strobilifera*

extracts in the type 2 diabetes model. In order to mimic the diet-induced diabetes incidence, rats are initially fed with a high-fat diet (HFD) for few weeks to produce dyslipidemia and pancreatic stress condition will be induced by using streptozotocin (STZ). Since, oxidative and nitrosative stress plays an important role in the pancreatic beta-cell damage and development of diabetes, therapeutic agents or plant extract having the potential to decrease these stress conditions may be a possible approach to protect pancreatic beta cells and prevent or reverse diabetes mellitus.

MATERIALS AND METHODS:

Materials: The present study was executed at the Department of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal. Collection and processing of plant materials whole plant of *Flemengia strobelifera* were obtained and authenticated by a taxonomist and plant voucher specimen number 991. Total cholesterol and triglyceride biochemical assay kits were procured from accurex (Mumbai, India). Streptozotocin (STZ), thiobarbituric acid (TBA), malondialdehyde (MDA), hematoxylin & eosin stains were purchased from sigma (Bangalore, India). Ellmann's reagent and Griess reagents were purchased from Hi-Media (Mumbai, India). All the chemicals used in this study were of research-grade quality procured from local suppliers.

Preparation of *Flemengia strobilifera* Extraction: The whole plant were cut into small pieces, dried at room temperature until completely dry. The dried plant's materials were ground by mechanically into a fine powder and sieved through a 40mm mesh sieve. The obtained powder was kept in airtight plastic bags. 100 gm of each powdered plant sample was extracted with 400ml of methanol with occasional shaking for 7 days by maceration.

The extract was filtered decanted into a clean conical flask and sieved through a Whatman filter paper into another conical flask. The methanolic extract of the whole plant was dispersed in 1000 ml of water separately and fractioned with toluene, ethyl acetate, and n-butanol. The solvents were removed from the fractions under reduced pressure to yield the corresponding extract. The toluene fraction was filtrated, dried in rotavapour and used for further studies.

Animals: Animal experiments were performed in accordance with the CPCSEA guidelines of the government of India. Animal protocols were approved by the institutional animal ethics committee (IAEC/10/UCPSC/KU/2016). Male Sprague Dawley (SD) rats were used for the high-fat diet (HFD) plus STZ (HFD+STZ) induced type II diabetes model and for evaluation of the protective effect of *Flemengia strobelifera* extract.

Animals were acclimatized to the experimental conditions one week before the experiment. Animals were maintained at standard conditions, 12 h day/light cycle, 50-60% relative humidity, food and water supplied as *ad libitum*.

Experimental Design:

Group 1: Normal control- animals fed with standard rat chow diet.

Group 2: HFD+STZ group- animals were first two weeks given only HFD and another 4 weeks HFD+STZ was given (STZ single administration (35 mg/kg, i.p.) on 15th day that is 2 weeks post HFD feeding).

Group 3: Low dose of the extract-treated group- HFD and HFD+STZ was given similarly and extract treatment was given 1 day post-STZ administration onwards till the end of study at the dose of 10 mg/kg/day, orally.

Group 4: Mid dose of the extract-treated group: HFD and HFD+STZ was given similarly and extract treatment was given 1 day post-STZ administration onwards till the end of the study at the dose of 30 mg/kg/day, orally.

Group 5: High dose of the extract-treated group: HFD and HFD+STZ was given similarly, and extract treatment was given 1 day post-STZ administration onwards till the end of the study at the dose of 100 mg/kg/day, orally.

Group 6: Standard Metformin treated group- HFD and HFD+STZ were given similarly and Metformin treatment was given 1 day post-STZ administration onwards till the end of the study at the dose of 10 mg/kg/day, orally.

Induction of Type II Diabetes: For induction of type II diabetes, male SD rats were initially fed

with high-fat diet for 2 weeks (pre-experimental period), followed by low dose of streptozotocin (STZ) 35 mg/kg was injected intraperitoneally (i.p) on 2 weeks post-HFD as per the well-established method [9].

Evaluation of Diabetogenesis in Rats: The incidence of diabetes was evaluated by estimating the plasma glucose levels every week. The blood glucose levels were measured by automatic glucometer by collecting the drop of blood from the rat tail. Rats with blood glucose levels >250 mg/dl were considered as diabetic and the percentage of diabetes was calculated [23].

Measurement of Pancreatic Weights: After completion of 4 weeks of the post-STZ experimental period (initial 2 weeks on HFD is considered pre-experimental period), on day 28 (after 4 weeks), Animals were sacrificed by a high dose of anesthesia and pancreas from each animal was collected carefully and wet weight of pancreas were recorded [25].

Oral Glucose Tolerance Test (OGTT): After completion of 4 weeks of the experimental period, a subset of animals from all the experimental groups was utilized to perform an oral glucose tolerance test (OGTT). Animals were fasted overnight and administered with 2g/kg dose of glucose orally, following glucose challenge; blood glucose analysis was performed by using glucometer at 0, 15, 30, 90 and 120 h post glucose administration. The time versus blood glucose levels was plotted to evaluate the glucose disposal behavior of animals and the effect of treatment on glucose intolerance [32].

Estimation of Biochemical Parameters: The plasma profile for metabolic parameters like triglycerides and total cholesterol in the plasma was estimated in all the experimental groups. These estimations done by using commercially available biochemical kits procured from Accurex, Biomedical (Mumbai, India) [24, 32].

Estimation of Oxidative and Nitrosative Stress Parameters in Pancreas: After completion of the study (28 days), animals were sacrificed, pancreas were isolated, and weights were recorded. In the pancreatic tissue the oxidative stress marker like malondialdehyde (MDA) levels, antioxidant marker glutathione (GSH) levels and estimation of tissue nitrosative stress, nitric oxide levels were measured [25, 27].

Estimation of MDA Levels in Pancreas: Pancreas were homogenized in 5 volumes of ice-cold PBS (pH 7.4) by using tissue homogenizer. The total homogenate was used for the malondialdehyde (MDA) estimation the colorimetric method.

Estimation of GSH Levels: Reduced glutathione (GSH) levels were measured in the supernatants of the tissue homogenates as per the previous method with some modifications [13, 14].

Estimation of Nitric Oxide (NO) Levels in Pancreatic Tissue: After homogenizing the pancreatic tissue, the total homogenate was centrifuged at 10000 rpm and the supernatant was used for the NO estimation according to the Griess method [15, 16].

Histopathological Evaluation of Pancreas: After sacrificing the animals, part of the pancreatic tissue was removed and fixed in 10% formalin saline. Formalin-fixed tissues were processed according to the standard histological tissue processing [17].

Statistical Analysis: All the experimental values were expressed as mean ± standard error of the mean (SEM). The statistical significance among the groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. P-value <0.05 is considered as statistically significant.

RESULTS AND DISCUSSION:

Effect of Extract Treatment on Blood Glucose Levels: The blood glucose levels levels during the 4 weeks of experimental periods in all the study groups, at the basal levels (2 weeks post HFD feeding), there was a mild 10-20% increase in the blood glucose levels in all the HFD fed animals, it is expected, due to high energy content resulted in mild hyperglycemia. Upon STZ administration, a significant increase in the blood glucose levels was observed in HFD+STZ control groups compared to normal control animals. The glucose levels were found to keep on increased until the experimental period of 4 weeks (28 days). Interestingly, the hyperglycemic condition observed in diabetic control animals was dose-dependently decreased in

extract-treated animals. The high dose of toluene extract at 100 mg/kg produced a highly significant reduction in the blood glucose levels by the end of 28 days of treatment. The high dose of extract exhibited hypoglycemic effects similar to standard drug Metformin. Low and mid doses of extract treatment resulted in mild to moderate control in blood glucose levels. **Fig. 1** explains the blood glucose profile of different groups during 28 days post-STZ induction and effect of extract and standard drug on glucose profiles.

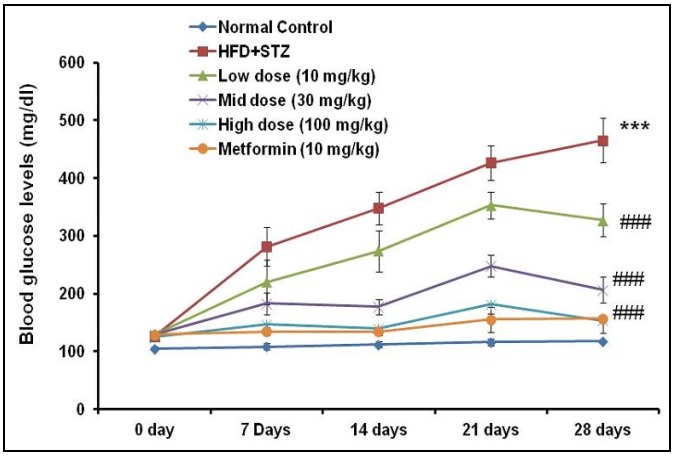

FIG. 1: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON GLUCOSE LEVELS EVALUATED IN HFD+STZ INDUCED TYPE II DIABETES MODEL. Data were represented as (Mean ± SEM) (n=6). *** P<0.001 *vs.* normal control group and ### P<0.001 *vs.* HFD+STZ group.

Effect of Extract Treatment on Diabetogenesis and Diabetes Incidence: Fig. 2 clearly demonstrates that at the basal levels (2 weeks post HFD feeding) none of the experimental animals were found to be diabetic. The average blood glucose levels were found to be way below the diabetic mark (250 mg/dl). That means, despite high calorie diet feeding the diabetic condition is not achieved in all these animals. 7 days post STZ induction; we can clearly see the biggest jump in the percentage of diabetes more than 80% of animals in untreated HFD+STZ animals. The percentage of diabetes was found to be 100% by 2 weeks post STZ induction and the same level was maintained up to 28 days.

Interestingly, treatment with extract at all three dose levels has shown a reduction in the percentage of diabetes with the best reduction in the diabetes percentage in high dose treated animals (80% reduction in the diabetes chances). When we compiled the final time point (28 days) incidence of diabetes, it is obvious that extract treatments dose-dependently decreased in the incidence of diabetes, **Fig. 3**.

The high dose extract-treated group has demonstrated a significant reduction in the incidence of diabetes, which is similar in extent compared to standard drug metformin.

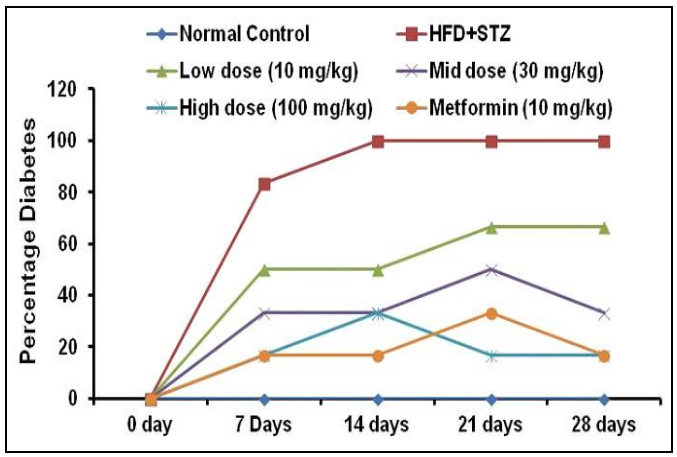

FIG. 2: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON DIABETOGENESIS EVALUATED IN HFD+STZ INDUCED TYPE II DIABETES MODEL. Data were represented as Mean+ SEM of 6 animal data. Animals with glucose value > 250 mg/dl were considered diabetic.

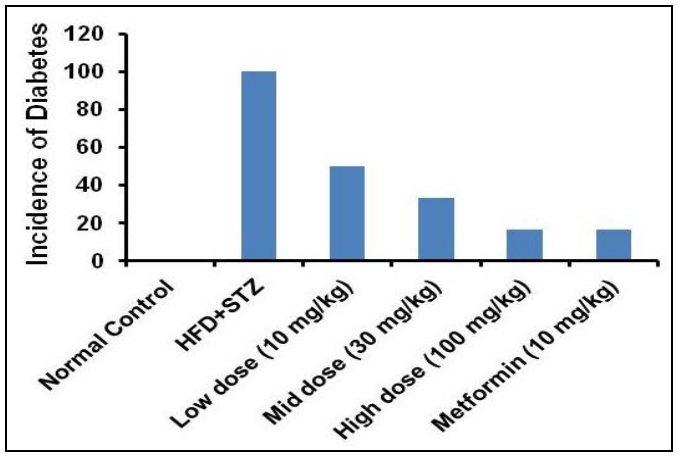

FIG. 3: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON THE INCIDENCE OF DIABETES AT THE END OF 28 DAYS EVALUATED IN HFD+STZ INDUCED TYPE II DIABETES MODEL. Data were represented as (Mean+ SEM) of 6 animal data. Animals with glucose value > 250 mg/dl were considered diabetic.

Effect on OGTT: Since OGTT is performed to evaluate the status of glucose intolerance and glucose disposal behavior of animals. It is observed from the blood glucose profile that upon glucose

challenge the levels were greatly increased in HFD+STZ animals compared to control animals. The glucose values were touched close to 600 mg/dl within 15 min post glucose challenge. Moreover, these glucose levels have not come down to normal levels after 90 min, in contrast, the glucose levels have come down to normal levels in control animals, which indicate the presence of glucose intolerance in HFD+STZ animals compared to normal control animals. Interestingly, extract treatments have resulted in significant effects on glucose profiles with dose-dependent improvement in glucose disposal behavior. A high dose of extract has normalized the glucose intolerance close to normal control animals. A similar kind of improved glucose tolerance was observed in metformin-treated groups **Fig. 4**.

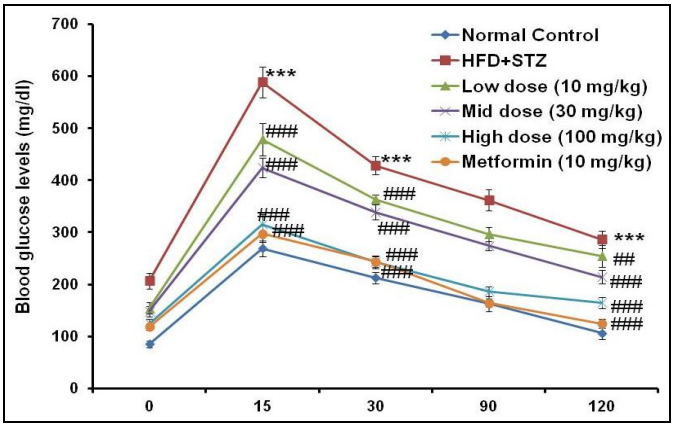

FIG. 4: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON BLOOD GLUCOSE LEVELS AT THE END OF 28 DAYS EVALUATED IN HFD+STZ INDUCED TYPE II DIABETES MODEL. Data were represented as the mean of 6 animal data. Animals with glucose value > 250 mg/dl were considered diabetic. Data were represented as mean ± SEM (n=6). *** P<0.001 *vs*. normal control group, ## P <0.01 and ### P<0.001 *vs*. HFD+STZ group

Effect on Pancreatic Weights: There was a significant reduction in the pancreatic weights in diabetic control animals (HFD+STZ) compared to non-diabetic control animals. Approximately 32% reduction in the pancreatic weight was observed in diabetic control animals. All three doses of extracts produced a trend of increased pancreatic weights dose-dependently **Fig. 5**.

Surprisingly, a high dose of extract produced 28% recovery of pancreatic tissue mass with a net pancreatic mass of only 4 lesser than that of normal control animals.

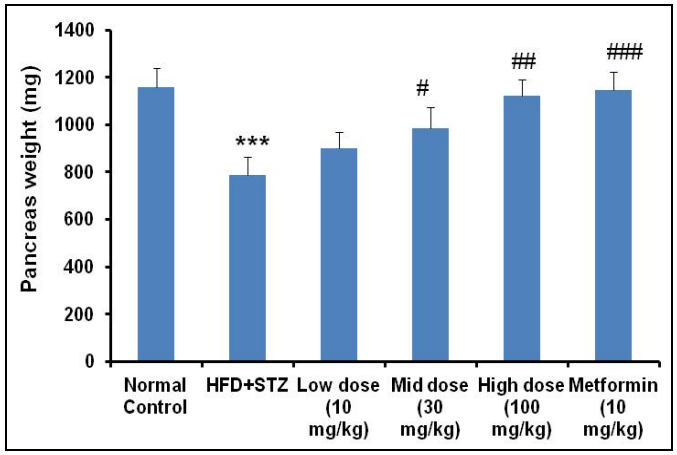

FIG. 5: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON PANCREATIC WEIGHT AT THE END OF 28 DAYS EVALUATED IN HFD + STZ INDUCED TYPE II DIABETES MODEL. Data were represented as mean ± SEM (n=6).). *** P<0.001 *vs*. normal control group, # P<0.05, ## P <0.01 and ### P<0.001 *vs*. HFD+STZ group

Effect of Extract on Pancreatic Oxidative Stress: The MDA levels which are markers of lipid peroxidation induced oxidative stress are greatly increased in HFD+STZ control pancreatic tissues compared to non-diabetic control pancreas. There was a 2.52 fold increase in the MDA levels in diabetic control groups compared to normal control animals.

Further, treatment with plant extract produced 1.28, 1.66, 2.16 fold reduction, respectively in 10, 30 and 100 mg/kg groups compared to diabetic control pancreatic tissues. Moreover, metformin treatment also produced a 2.2 fold reduction in the MDA levels **Fig. 6**.

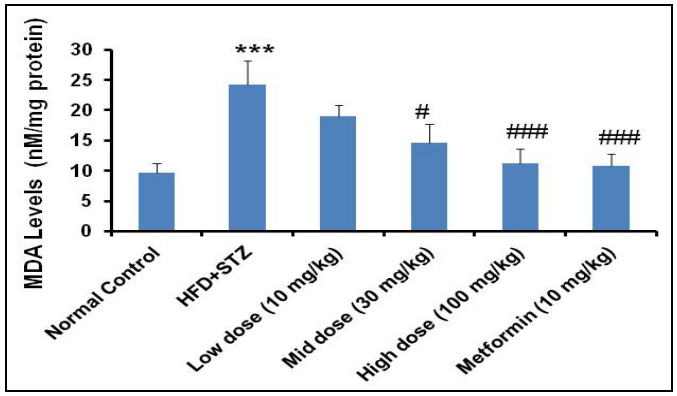

FIG. 6: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON PANCREATIC LIPID PEROXIDATION (MDA) LEVELS AT THE END OF 28 DAYS EVALUATED IN HFD+STZ INDUCED TYPE II DIABETES MODEL. Data were represented as mean ± SEM (n=6). *** P<0.001 *vs*. normal control group, # P<0.05 and ### P<0.001 *vs*. HFD+STZ group.

On the other hand, the endogenous antioxidant GSH was found to be 2.35 folds significantly decreased in HFD+STZ control pancreatic tissues compared to normal chow diet-fed control animals. Treatment with extract at 10, 30 and 100 mg/kg dose resulted in 1.25, 1.64 and 2.09 fold increase in the GSH levels. The high dose of extract produced a similar kind of increase like standard drug metformin **Fig. 8**.

Effect of Extract on Pancreatic Nitrosative Stress: The NO levels which is a marker of reactive nitrogen species status, suggested that there is an increase in nitrosative stress in diabetic control pancreas compared to non-diabetic control animals. There was a 2.53 fold increase in the NO levels in pancreatic tissues of HFD+STZ treated groups compared to normal pancreas. Further, treatment with extracts dose-dependently produced a reduction in pancreatic nitrosative stress, with a high dose group producing 2.14 fold reduction in the NO levels compared to diabetic control pancreas **Fig. 7**.

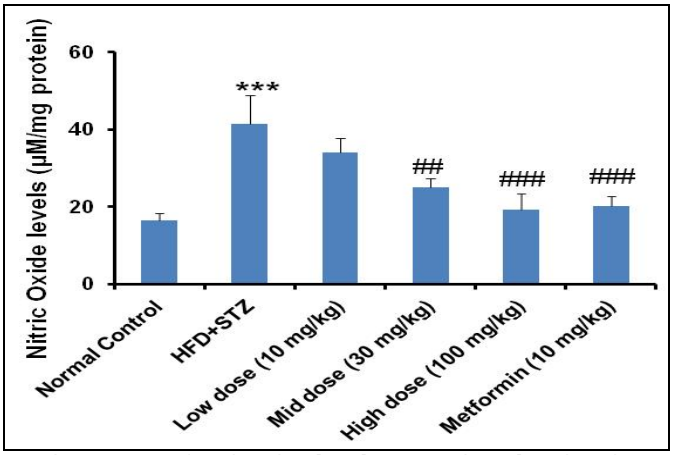

FIG. 7: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON PANCREATIC NITROSATIVE STRESS (NITRIC OXIDE) LEVELS AT THE END OF 28 DAYS EVALUATED IN HFD+STZ INDUCED TYPE II DIABETES MODEL. Data was represented as mean ± SEM (n=6). *** P<0.001 *vs.* normal control group, ## P <0.01 and ### P<0.001 *vs.* HFD+STZ group.

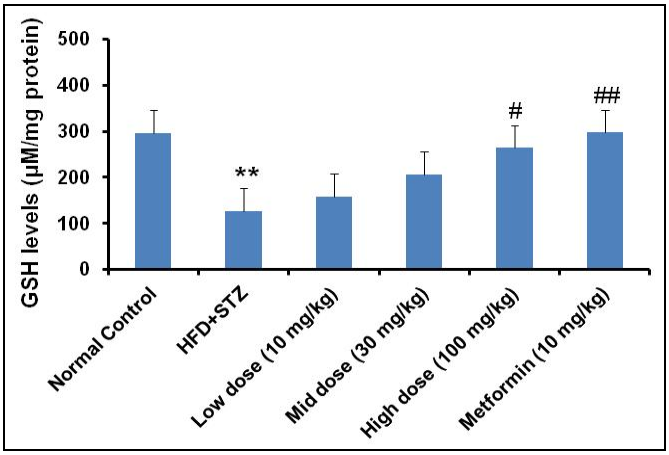

FIG. 8: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON PANCREATIC ANTIOXIDANT (GSH) LEVELS AT THE END OF 28 DAYS EVALUATED IN HFD+STZ INDUCED TYPE II DIABETES MODEL. Data was represented as mean ± SEM (n=6). ** P<0.01 *vs.* normal control group, # P <0.05 and ## P<0.01 *vs.* HFD+STZ group.

Effect of Extract on Plasma Lipid Profiles: The plasma triglyceride and total cholesterol levels were found to be greatly and significantly increased in HFD+STZ control animals compared to normal chow diet-fed control animals. There was a 2.17 and 2.56 fold significant increase in the triglyceride and total cholesterol levels in HFD+STZ animals compared to control animals. After 4 weeks of treatment with plant extracts resulted in a dose-dependent significant decrease in the triglyceride levels, **Fig. 9A**.

Similarly, the total cholesterol levels were also significantly decreased in mid (1.57 fold) and high (1.75 fold) doses of extract-treated animals compared to untreated diabetic control groups **Fig. 9B**. The high dose extracts treated animals exhibited lipid profiles that are almost close to metformin-treated animals.

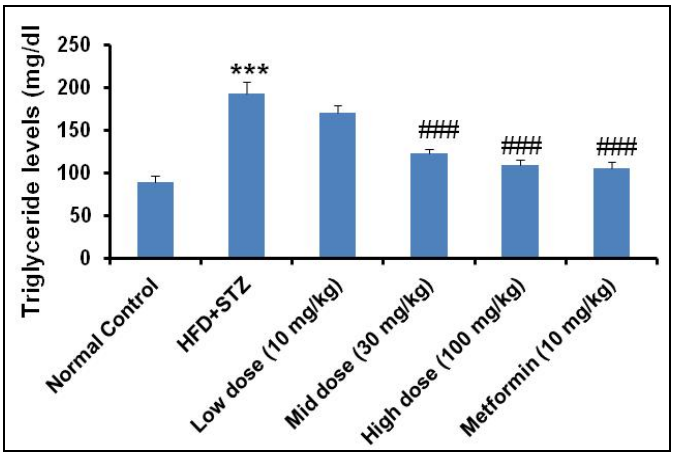

FIG. 9A: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON PLASMA TRIGLYCERIDE LEVELS AT THE END OF 28 DAYS EVALUATED IN HFD+STZ INDUCED TYPE II DIABETES MODEL. Data were represented as Mean ± SEM (n=6). *** P<0.001 *vs.* normal control group and ### P<0.001 *vs.* HFD+STZ group

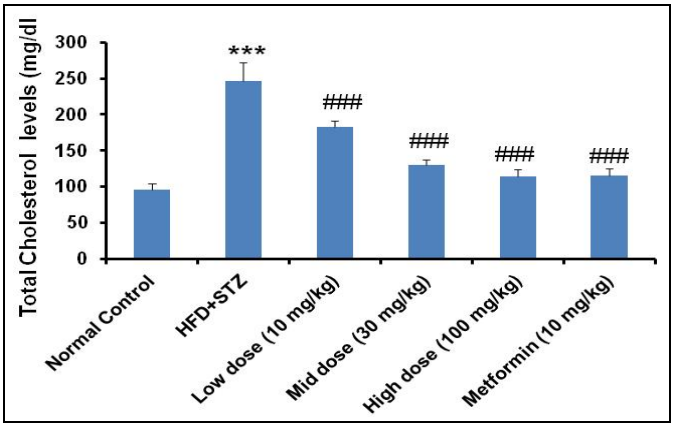

FIG. 9B: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON PLASMA TOTAL CHOLESTEROL LEVELS AT THE END OF 28 DAYS EVALUATED IN HFD+STZ INDUCED TYPE II DIABETES MODEL. Data were represented as mean ± SEM (n=6). *** P<0.001 *vs*. normal control group and ### P<0.001 *vs*. HFD+STZ group

Effect of Extract on Pancreatic Histology: The Hematoxylin and Eosin (H&E) staining performed on pancreatic tissues indicated degenerated and highly inflamed pancreatic histological features in diabetic control pancreas with severe infiltration of inflammatory cells into pancreatic tissues. Compared to HFD+STZ treated pancreatic tissues, the normal non-diabetic animals demonstrated clearly normal pancreatic beta-cell and acinar cell histological features with no evidence of beta-cell degeneration and inflammation. In diabetic control groups, the pancreatic histology demonstrated increased ductular fibrosis along with the proliferation of ductular epithelial cells. Treatment with plant extract produced dose-dependent amelioration of damaged histological features. In treatment groups, there was no evidence of pancreatic beta-cell degeneration observed. Similar kind of clean and normal histological features was observed on standard anti-diabetic drug metformin-treated animals.

The number of pancreatic beta cells and the average size of beta-cell and pancreatic cell areas were enormously decreased in HFD+STZ groups compared to non-diabetic control pancreas. Treatment with extracts produced progressive and dose-dependent improvement in the pancreatic beta-cell number and beta-cell sizes **Fig. 10**.

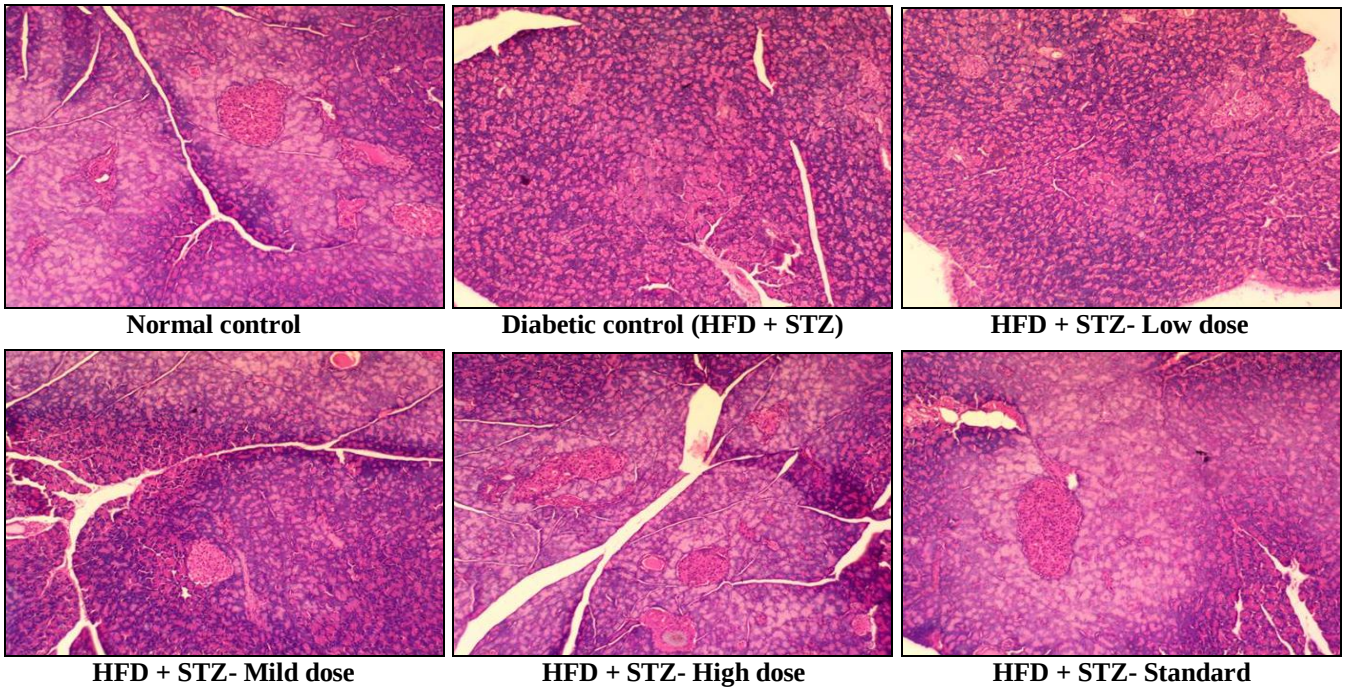

FIG. 10: EFFECT OF ORAL ADMINISTRATION OF *FLEMENGIA STROBILIFERA* EXTRACT ON HISTOLOGICAL FEATURES OF PANCREAS (H&E STAINING) EVALUATED IN HFD+STZ INDUCED TYPE II DIABETES MODEL. NORMAL CONTROL: GLANDULAR PANCREAS CONTAINING ISLETS CELLS APPEARED NORMAL COLLECTING DUCTS IN THE GLANDULAR PANCREAS APPEARED NORMAL, HFD+STZ: SEVERE DEGENERATION OF ISLETS CELLS IN GLANDULAR PANCREAS AND MODERATE TO SEVERE DUCTULAR FIBROSIS ALONG WITH PROLIFERATION OF DUCTULAR EPITHELIAL CELLS, LOW DOSE: MILD TO MODERATE BETA CELL DEGENERATION, MID DOSE: MILD TO MODERATE DEGENERATION OF BETA CELLS NOTICED IN THE ISLETS OF PANCREAS, HIGH DOSE: GLANDULAR PANCREAS CONTAINING ISLETS CELLS APPEARED NORMAL AND HFT+STZ WITH STANDARD METFORMIN: APPEARED NORMAL BETA CELLS IN THE ISLETS HAVE APPEARED NORMAL AND NO DEGENERATION NOTICED IN THE CELLS.

Diabetes is one of the leading causes of morbidity and mortality globally [18]. Due to modern lifestyles and increased industrialization, people are habituated to sedentary lifestyles, which resulted in an increased risk of obesity and metabolic syndrome [19]. Since, type II diabetes accounts for more than 90% total diabetes cases, in the current study we have made an attempt to evaluate the protective effects of *Flemengia strobilifera* extract in an experimental model of type 2 diabetes [20, 21]. The *Flemengia strobilifera* extract found to possess promising antioxidant properties evaluated by DPPH assay. Based on these in vitro antioxidant effects, the pancreatic beta-cell protective effect of the extract was evaluated in HFD+STZ induced type 2 diabetes model. Initially, animals were fed with HFD for weeks to introduce metabolic syndrome like insulin resistance condition, with mild hyperglycemia and full-fledged dyslipidemia [22]. The HFD+STZ model unique non-genetic animal model which is a widely used type 2 diabetes model for pharmacological screening. Once the hyperinsulinemia condition was observed in HFD fed animals, the pancreatic beta cells are under severe stress conditions to produce higher levels of insulin to maintain blood glucose levels. In such stress conditions, the low dose of STZ (35 mg/kg) can cause progress beta-cell damage which may mimic the human type 2 diabetic condition [6, 9].

In our model also we have seen severe damage to pancreatic beta cells within 1 week post-administration of STZ, though the dose of STZ is low, it still caused extensive pancreatic beta-cell damage due to co-existing metabolic syndrome-like condition [23]. As a result of continuous beta-cell damage, plasma glucose levels were progressively increased in HFD+STZ control animals. The plasma glucose levels were found to be significantly decreased in plant extract treated animals; it is clear from the figure that extracts efficiently controlled the hyperglycemic conditions in a time-dependent and dose-dependent manner. Similarly, the percentage of diabetes induction and diabetes incidence was substantially decreased in plant extract treated animals compared to untreated diabetic control animals. The possible explanation for this promising effect is the protection of pancreatic beta-cell damage by active constituents present in extracts [24]. A similar kind of pancreatic beta-cell protection was demonstrated with different plant extracts and their active ingredients [25]. Since pancreatic beta cells are degenerated or damaged in HFD+STZ treated an animal, the net pancreatic weights were significantly (almost 33% decrease) decreased. That means the increased pancreatic beta-cell damage due to HFD and STZ resulted in severe beta-cell crisis and severe hyperglycemia condition. Interestingly, chronic oral administration of *Flemengia strobilifera* extract produced encouraging beta cell-protecting hypoglycemic effects. These antihyperglycemic effects are comparable to the standard antidiabetic metformin-treated animals. The data from glucose levels and diabetes incidence clearly suggest that *Flemengia strobilifera* extract has a protective role against HFD+STZ induced pancreatic damage. Since plant extracts are protecting the pancreatic beta cells; the OGTT also further demonstrated improved glucose tolerance. The possible mechanism for this anti-diabetic effect of *Flemengia strobilifera* extract is its antioxidant and anti-inflammatory properties [26]. It is clear from the MDA analysis that there is an enormous increase in the oxidative stress in the diabetic control animals, several studies also reported increased oxidative stress and in metabolic syndrome conditions and STZ is known to produce an abundant increase in oxidative stress conditions in pancreatic tissues [27]. In similar lines, previous studies also demonstrated the protective effects of plant-derived compounds against pancreatic tissue damage [28].

In addition to increased oxidative stress, the endogenous antioxidant levels also severely compromised in diabetic pancreatic tissues, which also observed in our current study in which GSH levels were found to be decreased many-fold in HFD+STZ control pancreatic tissues. Moreover, treatment with *Flemengia strobilifera* extract resulted in a dramatic improvement in oxidative stress with significant elevation of endogenous antioxidant GHS. In addition, nitrosative stress also reported playing a crucial role in pancreatic damage, which might be the case in our current study, where, we had seen high NO levels in diabetic control animals, moreover, extract treatment resulted from promising effects in the nitrosative stress conditions. The possible source of increased NO levels in pancreatic tissues of diabetes groups is the overexpressed status of inducible nitric oxide synthase (iNOS), which

might be the possible mechanism for elevated nitrosative stress [29]. Moreover, *Flemengia strobilifera* extract produced a notable reduction in the NO levels. Since NO reacts with superoxides to form peroxynitrites, which are highly reactive free radical agents, it is important to control both oxidative and nitrosative stress in order to control the oxidative stress-mediated pancreatic beta-cell damage [30]. These extracts seem to be producing beta cell protection by controlling oxidative and nitrosative stress. Based on the biochemical profiles in the pancreas and plasma biochemical parameters, it is evident that there is an increased glucose level which is mainly due to the destruction of pancreatic tissue.

The histological evaluations also clearly suggest the pathological degeneration of pancreatic tissues, with associated pancreatic inflammatory cell infiltration and extracellular matric accumulation. Fortunately, treatment with *Flemengia strobilifera* extract resulted in notable improvement in the histological features of pancreatic tissues. Therefore, the active constituents present in *Flemengia strobilifera* extracts might be mainly responsible for pancreatic protection, which translated into decreased blood glucose levels and decreased diabetes incidence in extract-treated animals compared to untreated diabetes control animals. Since both HFD feeding and type 2 diabetes conditions produce dyslipidemia, we have evaluated the lipid profiles [31]. It is obvious to note that plasma total cholesterol and triglyceride levels were significantly increased in HFD+STZ control animals, due to such metabolic syndrome-like conditions, the body develops insulin resistance. There is a very good connection between increased lipid profiles and impaired glucose tolerance, which we have demonstrated through OGTT and found that diabetic control animals exhibit significant dyslipidemia and glucose intolerance [32]. Interestingly, treatment with *Flemengia syndrome-like* extracts resulted in a significant reduction in plasma triglyceride and total cholesterol levels and also produced significant improvement in glucose tolerance. Further, it is also possible to control the cardiovascular complications associated with type 2 diabetes by effectively controlling the lipid levels [33].

Thus, our extract might be effective in the management of diabetic complications mainly vascular complications. Further, studies are warranted to explore these pharmacological assumptions. Though we have demonstrated promising pancreatic protective and antidiabetic effects of *Flemengia strobilifera* extracts, the doses used in the current studies are in the relatively higher range, therefore, further studies may be required to overcome dose-related problems. Taken together our experimental evidence, it is possible to treat type 2 diabetes. Therefore, possibilities for clinical translation could be explored for further exploration of such an interesting plant extract with proven medical benefits.

CONCLUSION: Our results clearly demonstrated that the significant beneficial effects of *Flemengia strobilifera* extracts in protecting the pancreas from the high-calorie diet and STZ induced deleterious effects. More detailed molecular studies and isolation of active constituents from this plant may further provide vital information to explore for clinical translation.

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