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EFFECT OF *ACYRANTHUS ASPERA* ON HIGH-FAT DIET AND STREPTOZOTOCIN INDUCE DIABETIC RATS: ROLE OF OXIDATIVE STRESS, PPAR-A, GLUT-2 AND INSULIN SIGNALLING

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Keywords:

Achyranthes aspera, Anti-diabetic, Anti-obesity, Anti-oxidant

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ABSTRACT: Diabetes mellitus is a complex disorder where oxidative stress plays a crucial role in the development and progression of the disease. In the present work, the rat model evaluated pancreas and liver tissue damage caused by a high-fat diet and diabetic complications. Male Wistar rats were administered with HFD- corn oil orally for 28 days followed by streptozotocin (STZ) injection to induce diabetes on 29th day. The drug treatment groups were given low dose (250mg/kg) and high dose (500mg/kg) of hydroalcoholic extract of *Achyranthes aspera* (HEAA), and its therapeutic efficacy in HFD + diabetes model was compared with the standard drug metformin. Various antioxidant parameters were measured, including MDA, protein, GSH levels, SOD, and CAT activities. The histology of pancreas tissues was also analyzed using Haematoxylin, Eosin, and Masson-trichrome staining. The expression of insulin protein in the pancreas was studied by immunohistochemical technique. The effect of drugs on GLUT-2 and PPAR-α gene expression showed considerable improvement in the liver tissues of different groups. From the results, it can be seen that the anti-diabetic and antihyperlipidemic effect of HEAA seems convincing. HEAA can be used to manage diabetic complications associated with obesity.

INTRODUCTION: Diabetes mellitus is one of the most prevalent metabolic disorders and is expected to affect more individuals in the future. By 2040, 642 million people are expected to be affected with the same [1].

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There are a number of risk factors for type 2 diabetes mellitus, including family history, physical inactivity, sedentary lifestyle and ethnicity.

A high-fat diet is another risk factor linked to type 2 diabetes mellitus and is also connected with other conditions like cardiovascular disorders. Diets rich in carbohydrates and fat have been associated with conditions like type 2 diabetes mellitus, obesity, and cardiovascular disorders [1]. Oxidative stress and chronic inflammation also play a major role in developing diabetes mellitus. Oxidative stress helps

type 2 diabetes mellitus progression by promoting cellular dysfunctions in muscle, adipose, and pancreatic islet tissues [2]. It causes cellular dysfunctions through increased apoptosis, decreased β cell neogenesis, and mitochondrial dysfunction [3]. Oxidative stressors increase NF-κB signalling, which promotes inflammation and causes insulin resistance [2]. The liver expresses the GLUT-2 and PPAR-α genes, which influence metabolic glucose homeostasis.

The treatment for diabetes mellitus involves medications that are categorized intoα-Glucosidase inhibitors, sulfonylureas, and GLP-1 receptor agonists. It is associated with side effects like weight gain, hypoglycaemia, and other cardiovascular complications [3]. According to the World Health Organization, the public shows interest in using traditional medicine [4]. *Acythranthus aspera* is a tropical wild perennial herb and has been used as traditional medicine for decades [5]. It is commonly known as Agatha in Sanskrit and is predominantly found in India [4]. *Acythranthus aspera* seeds, roots and leaves are used in Ayurveda for vomiting, piles, dysentery and blood disease [6].

Achyranthes aspera, has antioxidant, anti-inflammatory and hepatoprotective activities.[7]Its therapeuticrole in hyperlipidemic and hypercholesteremic conditions have been proved[8]. It also has spermicidal activity, anti-arthritic and cardio-protective roles as well [9]. The effectiveness of the hydroalcoholic extract of *Achyranthes aspera* (HEAA) on diabetes in combination with high fat diet complications has not been studied. Therefore this study has been conducted on oxidative stress parameters, insulin protein expression, GLUT-2 and PPAR-α gene expression in a rat model.

METHODOLOGY:

***Achyranthes aspera* Root Hydroalcoholic Extract:** The *Achyranthes asper* root parts were collected from the field. A qualified Botanist performed the Botanical identification and authentication, and a specimen was deposited in the herbarium (Voucher No: 57403). One litre of 70% ethanol was used to immerse 100 g of root parts of the *Achyranthes aspera* for one month. The contents were filtered using muslin cloth, concentrated using a rotatory evaporator (Re-100pro Model, Neoscience Lab Pvt Ltd, Tamil Nadu), and then lyophilized. The yield percentage was 19.5%. The dried material was stored at 4 °C.

Animals: Wistar male rats weighing 150-200 g were used for this study. The rats were housed in polypropylene cages (3 per cage) with sterile paddy husk for a week to acclimate to the laboratory environment. The rats were fed on pellet meal (Biogen, Bangalore) and given filtered water *ad libitum* while being observed at the Centre for Laboratory and Animal Research (CLAR), Saveetha Medical College.

Ethics: The protocol was approved by the Institutional Animal Ethics Committee, Saveetha Medical College (SU/CLAR/RD/002/2021). Animals were housed and maintained according to the standards of the "Committee for control and Supervision of Experiments on Animals" (India). The bedding husk material was changed on a regular basis to maintain a hygienic environment.

Experimental Groups: A single dose of streptozotocin was administered intraperitoneally to induce hyperglycemia at a dose of 40 mg/kg following 5 weeks of induction with Vanaspati + coconut oil (3:2) as a high fat diet for 28days to induce hyerlipidemia. A low and high dose of HEAA (of 250 mg/kg and 500 mg/kg) was administered orally for five weeks. The therapeutic doses were given after streptozotocin and high-fat diet were administered. In this experiment, thirty-six male Wistar albino rats were employed. Six groups of animals were used (6 rats in each group). The rats in Group 1 were used as a control group 0.5 mL/kg normal saline was administered orally. The rats in Group 2 were provided with a single dose of Streptozotocin 40 mg/kg, intraperitoneally. The rat in Group 3 to Group 6 was administrated with high-fat diet, then Diabetes induction was done by a single dose of Streptozotocin 40 mg/kg intraperitoneally. Group 4 was given HEAA (250 mg/kg), Group 5 was given HEAA (500 mg/kg) and the Group 6 was given metformin (250 mg/kg) orally as a single dose, respectively.

Preparation of Tissue Homogenate: In Tris - HCl buffer (0.1 M, 4°C, pH 7.4), the weighed pancreas and liver tissues were homogenized using a Potter -

Elvehjem homogenizer with a Teflon pestle run for 3 minutes at 600 rpm. It was then centrifuged at 3000 xg at 4°C for 10 minutes with Remi refrigerated centrifuge and taken for analyzing the antioxidant parameters.

Determination of MDA: To measure the oxidative damage the levels of Malondialdehyde in the tissues were quantified using the thiobarbituric acid reaction method. To measure the MDA level, a working solution comprising of thiobarbituric acid, trichloroacetic acid and 0.25 N hydrochloric acid was prepared.

250 μL tissue homogenate and 500 μL working solution were added to each sample, kept in boiling water for 10 min, and centrifuged for 10 min at 3000 rpm. Lastly, 200 μL of each supernatant was transferred to microplates, and the optical density of the samples was measured at 535 nm. The values of MDA are expressed as nmoll/gram protein.

Assay of SOD: The SOD activity depends upon the production of superoxide radicals by xanthine oxidase and xanthine, that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to make a red formazon dye. Mixed substrate (300 μL) was added to tissue homogenate (200 μL). The samples were mixed well and 75 μL xanthine oxidase was added to reactions. The absorbance was taken at 505 nm and the SOD activity was then measured and expressed in U/mg protein [10].

Assay of Catalase: About 10 μL of tissue homogenate was pipetted intesttube and added 100 μmol/mL of H_2O_2 in 0.05 mmol/L Tris-HCl buffer 7.0 pH and incubated for 10mins. The reaction was ended by promptly adding 4% ammonium molybdate, and the absorbance was measured at 410 nm. One unit of catalase activity was expressed as the quantity of enzyme needed to decompose 1 μmol H_2O_2 per min [11].

Determination of GSH: About 15 μL of tissue homogenate was added to 260 μL assay buffer (0.1 M sodium phosphate and 1 mM EDTA, pH: 8) and 5 μL Ellman reagents. Samples were kept at room temperature for 15 min, and the TNB^{2-} formation was quantified in a spectrophotometer by taking the absorbance of visible light at 412 nm[12].

Hematoxylin and Eosin Staining: For analyzing histopathology and for light microscopic study of pancreas, tissues were cut and processed for paraffin sectioning. Then the tissues were hydrated and dehydrated in sorted alcohol series. It was then cleared using xylene and chloroform, and then it was fixed in paraffin wax using rotary microtome, tissues sections were taken (10µm) out and kept overnight at room temperature. It was then de-paraffinised and moistened with descending alcohol concentrations followed by distilled water. The sections were stained using Haematoxylin and Eosin stain and then immersed in ascending alcohol concentrations. A permanent slide was prepared using a DPX mount. The slides were observed under a light microscope (Olympus microscope), and photomicrographs were taken using a Sony digital camera.

Masson Trichrome Staining: Weigert's iron hematoxylin stains the nuclei in black, Biebrich scarlet-acid fuchsin stains cytoplasm and muscle fibers in red and after treatment with phosphotungstic and phosphomolybdic acid, collagen is stained in blue with aniline blue [14]. The slides were brought to room temperature, and the tissue sections were surrounded with a liquid blocker (PapPen). The cryosections were fixed in 10% Formalin at room temperature for 1hr in the hood. The sections were fixed in Bouin's solution at room temperature overnight to intensify the colors and increase the contrast among the tissue components. The slides were rinsed under running tap water (18–26°C) for 1-2 minutes to remove the yellow color from sections. Then using deionized water (ddH_2O), these sections were rinsed. The same volume of Hematoxylin Solution A and B was mixed together and kept in Weigert's Iron Hematoxylin solution for 5 minutes to stain the nuclei dark. The sections were kept with Biebrich Scarlet-Acid Fuchsin Solution for 5 minutes to stain the fibers red. The permanent slide was prepared using a DPX mount. The slides were observed under a light microscope (Olympus microscope), and photomicrographs were taken using a Sony digital camera.

Immunohistochemical Evaluation of INS Expression: Immunohistochemical staining for insulin protein expression was performed. For this purpose, a HRP/DAB detection IHC kit was used

according to the manufacturer's protocol. Paraffin was removed in xylene and the sections were dehydrated through alcohol series. After two rinses in PBS for 5 min each, the endogenous peroxidase activity was removed by incubation in 3% hydrogen peroxide for 30 min at room temperature. The non-specific binding sites were blocked by incubation with normal goat serum (3 drops in 3% BSA in PBS) for 30 min. After antigen retrieval (100 × Citrate Buffer) for 20 min in a domestic pressure cooker and blocking non-specific binding sites with protein block, the sections were immune reacted with 10 µg/mL primary antibodies against INS (Sigma Aldrich Company, place, Country) overnight at 4°C, respectively. For negative controls, the sections were immersed in PBS instead of the specific antibody. After rinsing with PBS, sections were incubated with biotinylated anti serum (goat antiserum to rabbit IgG 1:50 dilution) for 60 min at room temperature. Then the sections were incubated in the working streptavidin HRP solution for 60 min at room temperature and washed in three changes of PBS. Finally, the sections were incubated with DAB-hydrogen peroxide for 30 minutes and washed in water, counterstained and viewed under a light microscope. The area of positive staining was measured in pixels using Image-Pro Software, which detected brown staining in the tissue. The average optical density (AOD) was defined as the percentage of positive area in the total area of tissue sections.

6.2.5 Gene Expression of GLUT2 and PPAR- α by qRT-PCR: Using TRIzol reagent, the RNA was isolated from the primary hepatocytes according to the manufacturer's protocol (Life Technologies). For RT-PCR, the first strand of cDNA was synthesized from 3 µl of total RNA using random hexamers and Superscript II reverse transcriptase (Life Technologies). In a total volume of 50 µl, one microliter of the reverse transcription reaction mixture was amplified with primers specific for GLUT2 and PPAR-α. Amplification cycles between 25 and 30 helped test the PCR's linearity. The amplification of the samples was done according to the test amplification profile, at 94°C for 30 s, 56°C for 30 s, and 72°C for 28 s. For the internal control for quality and quantity of RNA, β-actin was used. The rat's primers of internal control β-Actin were purchased from Sino Biologicals Inc., USA. The primers of PPARs were designed using the Premier 5.0 primer based on the rat PPARs cDNA sequence in gene bank. The PCR primers used were as follows: GLUT2 sense, 5′-GGCTAATTTCAGGACTGGTT-3′; GLUT2 antisense, 5′-TTTCTTTGCCCTGACTTCCT-3′. The PCR for PPAR-α contained 1 µM each of the sense primer (5′-TCA TCA AGA AGA CGG AGT CG-3′) and the antisense primer (5′-CGG TTA CCT ACA GCT CAG AC-3′) in a total volume of 50 µl, resulting in a 211-bp fragment. PCR was then visualized on a 1% agarose gel containing 5 µg/ml ethidium bromide. β-actin expression was examined in identical conditions as an internal control (sense primer, 5′-TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA-3′ and antisense primer, 5′-CTA GAA GCA TTT GCG GTG GAC GAT GGA GGG-3′).

Statistical Analysis: The results were presented as Mean ± SEM for six animals in each group for each parameter. The statistical comparison was done by using SPSS software. A significant variation was observed, mean values were related using one-way ANOVA. A p-value less than 0.05 were meant to be statistically significant.

RESULTS:

Effect of HEAA on MDA and Protein Levels: From the graph **Fig. 1**, it can be seen that the MDA and protein levels of the rats in different groups were analyzed.

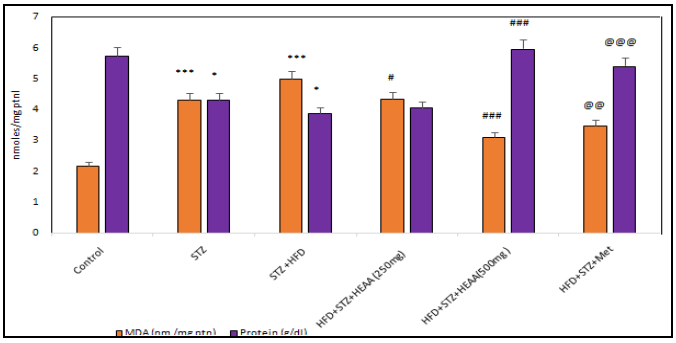

FIG. 1: MDA AND PROTEIN LEVELS OF THE RATS IN HEAA-TREATED STREPTOZOTOCIN AND HFD-INDUCED RATS. Data are expressed as mean ± SEM (n=6); ***p<0.001 vs control group. $^{\#\#\#}$p<0.001, $^{@}$p<0.05, $^{@@}$p<0.01, $^{@@@}$p<0.001 vs diabetic group.

In the present study, MDA, the oxidative stress marker, was found to p<0.001 significantly increased in the diabetes and HFD-induced group when compared to the control rats. HEAA (250mg)

and (500mg) showed ($p<0.05$) and ($p<0.001$) significantly lower level of MDA when compared with HFD and streptozotocin+HFD groups. The protein levels were increased in HEAA 250mg and also in HEAA 500mg treated rats and more reduction of MDA levels can be seen after the treatment with HEAA (500mg), very similar to that of metformin-treated rats.

Effect of HEAA on Antioxidant Enzymes: Analysis of antioxidants showed significantly decreased SOD, glutathione, and catalase activities in diabetes and HFD-induced group compared to the control group. The activities of enzymatic antioxidants SOD, CAT, protein, and non-enzymatic antioxidant, GSH was found to be increased in the rats treated with HEAA (500mg) similar to that of standard metformin drug when compared to diabetes and high-fat diet-fed groups **Fig. 2.**

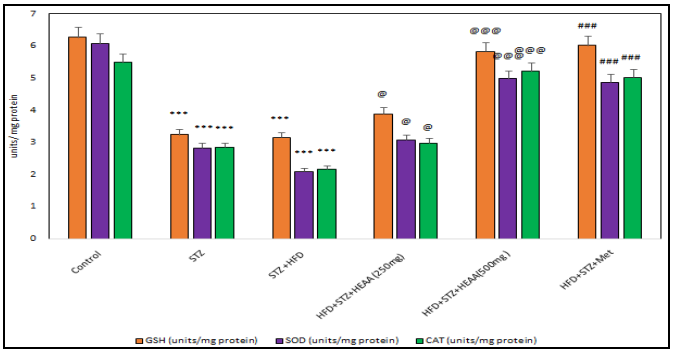

FIG. 2: GSH, SOD AND CAT ACTIVITIES OF THE RATS IN HEAA-TREATED STREPTOZOTOCIN AND HFD-INDUCED RATS. Data are expressed as mean ± SEM (n=6); $^{***}p<0.001$ vs control group. $^{###}p<0.001$, $^{@}p<0.05$, $^{@@@}p<0.001$, $^{###}p<0.001$ vs diabetic group.

Effect of HEAA on Pancreas Histology: The histology of the pancreatic tissues was analyzed and illustrated in **Fig. 3**. It can be seen from the histology report of the normal control rats depicting normal acinar cells **Fig. 3A**.

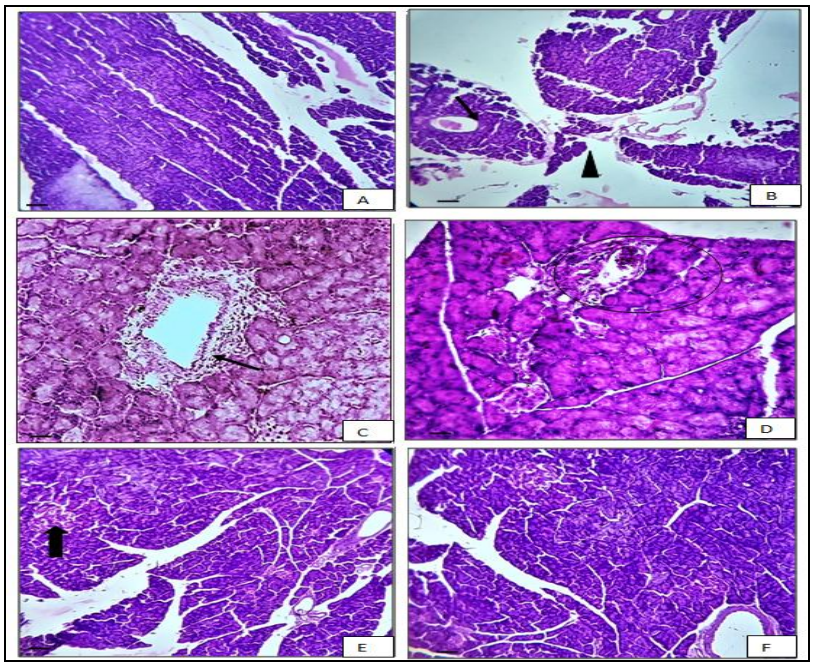

FIG. 3: HISTOLOGY REPORT OF PANCREATIC TISSUES IN HEAA TREATED STREPTOZOTOCIN AND HFD INDUCED RATS (10X), Scale bar - 50µm**.** 3A- Normal Control, 3B- Diabetic rats;3C- STZ + HFD, 3D- HEAA (250mg), 3E - HEAA (500mg), 3F - Metformin treated.

In diabetic and high fat-induced rats, fatty acids vacuolation inside islets of Langerhans and interstitial edema **Fig. 3C** was visualized. This indicates the damage caused to the pancreas of HFD-induced diabetic rats. HEAA (250mg/kg) treated rats did not exhibit better changes **Fig. 3D** when compared with the metformin-treated group. HEAA (500mg/kg) diabetic-treated rats showed minimum inflammation and reduced fatty infiltration **Fig. 3E**. It can be seen that metformin-treated rats showed less number of fatty globules and less infiltration **Fig. 3F.** The control rats showed a normal degree of collagen fibers detected in the portal duct and portal tract area (black arrow mark) **Fig. 4A**, diabetic rats following high-fat induction showed(orange arrow mark) extensive intralobular collagen deposition and fibrosis apparently as intense blue-stained collagen fibers

content in the tissue **Fig. 4B** and **4C**. The diabetic rats treated with HEAA (250 mg/kg) group showed the appearance of interacinar collagen fibers **Fig. 4D**, HEAA (500mg/kg) **Fig. 4E** and metformin-treated group **Fig. 4F** showing minimal fibrosis formation and irregular interlobular duct **Fig. 4A-F.**

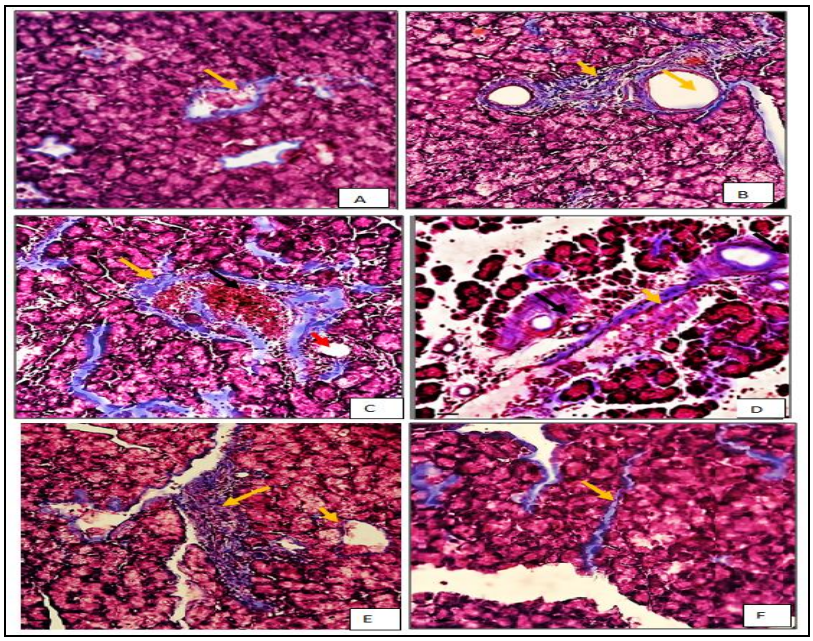

FIG. 4: HISTOLOGY REPORT OF LIVER TISSUES IN HEAA-TREATED STREPTOZOTOCIN AND HFD-INDUCED RATS (10X). Scale bar - 50µm**.** 4A- Normal Control, 4B- Diabetic rats;4C- STZ + HFD,4D- HEAA (250mg), 4E - HEAA (500mg), 4F - Metformin treated.

Effect of HEAA on Insulin Protein Expression: The immunostaining results of the pancreas tissues are depicted in **Fig. 5A-F**. The immune reactivity for insulin was well expressed in pancreatic tissue in normal control and metformin-treated groups **Fig. 5A** and **Fig. 5F** (arrow mark indicates dark brown color positive expression).

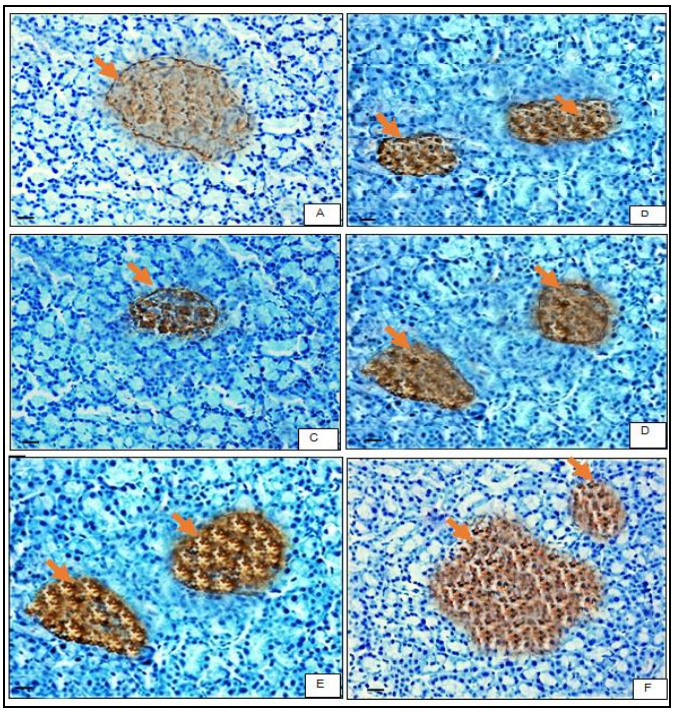

FIG. 5: IMMUNOSTAINING OF INSULIN PROTEIN EXPRESSION IN PANCREAS OF HEAA-TREATED STREPTOZOTOCIN AND HFD-INDUCED RATS. (Scale bar-25µm; magnification -200x).5A- Normal Control, 5B- Diabetic rats; 5C- STZ + HFD,5D- HEAA (250mg), 5E - HEAA (500mg), 5F - Metformin treated.

In diabetic rats **Fig. 5B** and diabetic + high fat induced group **Fig. 5C** immunostaining intensity of insulin was decreased in beta cell population of the tissues. However, less positive expression was seen in the diabetic-induced HFD rats treated with HEAA (250mg) **Fig. 5D**.

In HEAA (500mg/kg) treated rats **Fig. 5E**, the immune staining intensity of insulin was highly increased in beta cell population of pancreatic tissues. The INS protein expression was also quantified, and the test drug's significant effectiveness was depicted in **Fig. 6.**

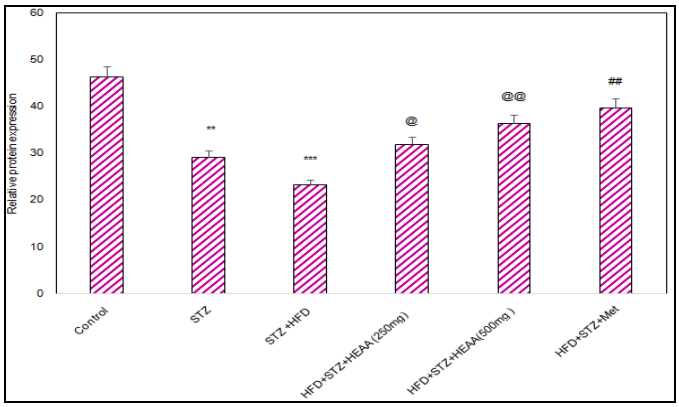

FIG. 6: QUANTITATIVE DATA FOR INS PROTEIN EXPRESSION IN HEAA-TREATED STREPTO-ZOTOCIN AND HFD-INDUCED RATS. VALUES ARE EXPRESSED IN INTENSITY UNITS. Data are expressed as mean ± SEM (n=6); *p<0.05;**p<0.01 vs control group. $^{@}$p<0.05, $^{@@}$p<0.01, $^{\#\#}$p<0.01 vs diabetic group.

Effect of HEAA on PPARα and GLUT2 Protein Expression: The gene expression levels of PPARα using qRT-PCR were analyzed in **Fig. 7.**

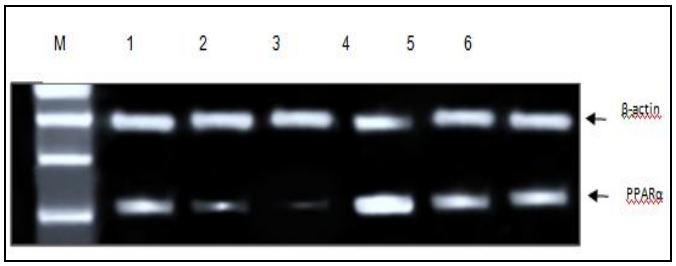

FIG. 7: GENE EXPRESSION LEVELS OF PPARA USING QRT-PCRIN HEAA TREATED STREPTOZOTOCIN AND HFD INDUCED RATS. Lane M- Marker lane (100-1000 bp); Lane 1 – Normal control; Lane 2 – Diabetic rats ; Lane 3 – Diabetic +HFD; Lane 4 – HFD+STZ+Metformin; Lane 5 – HFD+STZ+HEAA (250mg); Lane 6– HFD+STZ+HEAA (500mg).

PPARα gene expression was (p<0.001) significantly reduced in the diabetic and high-fat diet group compared with normal control group. HEAA 500mg/kg and metformin (standard drug) treated group showed (p<0.001) significantly increased expression as compared with diabetic and HFD group. The gene expression levels of PPARα were quantified and expressed in graph **Fig. 8.**

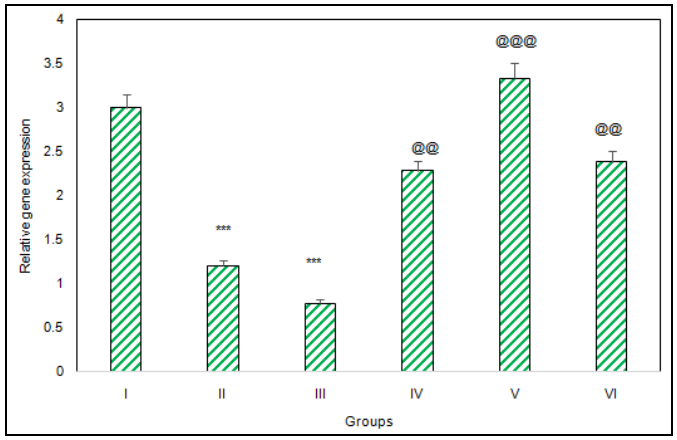

FIG. 8: SHOWS THE GRAPHICAL RE-PRESENTATION OF PPARA GENE EXPRESSION LEVELS FROM THE SCANNED IMAGE. Values are represented as Mean ± SD (n=3) Data are expressed as mean ± SEM (n=3); ***p<0.001 statistically significant as compared with Group I. $^{\#\#\#}$p<0.001, $^{@}$p<0.05,$^{@@@}$p<0.001 statistically significant as compared with Group II and III.

The gene expression levels of GLUT2 in liver tissue using qRT-PCR were analysed **Fig. 9**. GLUT2 gene expression was (p<0.001) significantly reduced in high fat diet induced diabetic group compared with normal control group.

HEAA 500mg/kg and metformin treated group showed (p<0.001) significantly increased expression as compared with diabetic and HFD group. The gene expression levels of GLUT2 were plotted as graph **Fig. 10.**

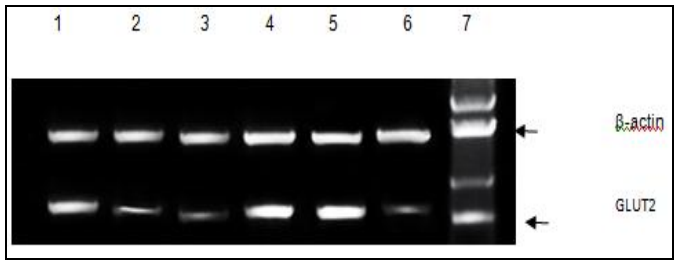

FIG. 9: GENE EXPRESSION LEVELS OF GLUT2 USING QRT-PCR IN HEAA TREATED STREPTOZOTOCIN AND HFD INDUCED RATS. Lane 1 – Normal control; Lane 2 – Diabetic group; Lane 3 – Diabetic +HFD group; Lane 4 – Diabetic +HFD+Met; Lane 5 – Diabetic +HFD+HEAA(500mg); Lane 6 – Diabetic +HFD+HEAA(250mg); Lane 7 –Marker Lane

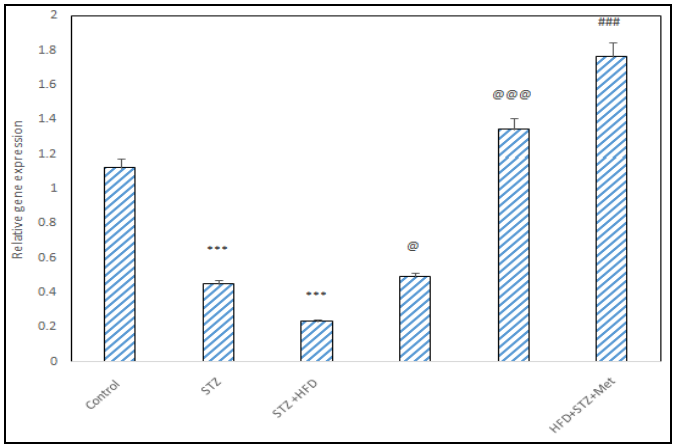

FIG. 10: SHOWS THE GRAPHICAL RE-PRESENTATION OF GLUT2 GENE EXPRESSION LEVELS FROM THE SCANNED IMAGE. Data are expressed as mean ± SEM (n=3); $^{***}p<0.001$ statistically significant as compared with control group. $^{###}p<0.001$, $^{@}p<0.05$,$^{@@@}p<0.001$ statistically significant as compared with diabetic and HFD group.

DISCUSSION: Oxidative stress has been shown to have a link with diabetes mellitus Newsholme *et al.,* 2016. Whenever there is an imbalance between the oxidants and antioxidants, a number of disorders like impaired cognitive function, hyperlipidemia linked with cardiovascular disease and diabetic complications occurs [13]. This indicates that the oxidative stress has to be managed in diabetes mellitus to reduce the pathological changes.

Oxidative stress has been linked to a number of pancreatic disorders including acute pancreatitis. An increased production of ROS is known to damage pancreatic cells by inhibiting the plasma lemmalcalcium ATPase pump. This in turn causes increased levels of calcium, thereby leading the pancreatic cells to necrosis. ROS also can induce apoptosis in the pancreatic cells. Apart from which, ROS amplifies the inflammatory pathway via MAPK pathway. Additionally increased ROS in the pancreatic tissues causes an alteration in the levels of xanthine oxidase and nitric oxide synthase. ROS can recruit more pro-inflammatory cytokines in the pancreatic tissues, thereby promoting inflammation [14]. Increased adipose tissues, especially in the abdominal region, causes a variety of molecular mechanisms to occur which in turn results in increased insulin resistance, chronic inflammation, blood pressure and lipid dysfunction [15]. These factors cause the development of certain disorders and involved in causing cellular damage which in turn affects the organs [16, 14]. All these factors contribute to oxidative stress, inflammation, obesity and diabetes mellitus type 2 are interrelated and can cause various complications.

This study shows that MDA, a marker for oxidative stress 14, has been increased in high-fat diet rats induced with diabetes. Enzymes like SOD, CAT, and GSH, which play a major role in maintaining the oxidative stress of the cells, have been decreased in rats with both high-fat induction and diabetes mellitus. Whereas administration of HEAA to high-fat diet rats induced with diabetes, there was a significant increase in their activities, indicating that these enzymes play a major role in reducing oxidative stress.

The histology report of the pancreatic tissues shows that the oxidative stress was reduced upon *Achyranthes aspera* treatment compared to rats induced with diabetes mellitus and high fat diet. Diabetic and high fat diet rats showed pale islet of Langerhans, fatty lobules, cytoplasmic vacuolation inside islets of Langerhans and interstitial edema in between pancreatic acini. Minimum inflammation and reduced fatty infiltration were noted when the dose of *Achyranthes aspera was*, whereas metformin treated rats showed less number of fatty globules and minimal infiltration. When the pancreatic cells are exposed to prolonged stress or inflammation, it has been found that β-cells undergo differentiation which in turn leads to the death of the β-cells [17]. In diabetes mellitus associated with hyperlipidemia, the pancreas undergo atrophy which further confirms that the molecular mechanisms leads to histological changes in the pancreas [18]. Apart from all these, there is evidence that there are alterations in the insulin expression caused by the alterations in the receptors in diabetes mellitus type 2 [19].

The results obtained were consistent with the results of other studies. A similar study done by Adeyi *et al.* revealed that there are histological changes in the pancreas of diabetic rats where the Islet of Langerhans had severe vacuolations and degeneration [20]. Furthermore, another study proved similar results concerning the Islet of Langerhans degradation and vacuolationin pancreas of diabetic rats and upon administration of a chronic high fat diet [21].

The insulin secretagogue action might be due to the progress of glucose transport gene (GLUT-2) and its effect on carrying proteins and fatty acid metabolism. Due to an increase in insulin expression in HEAA-treated rats and because of the GLUT-2 gene translocation to the cell membrane, the analysis has been made to determine the effect of HEAA treatment on liver tissue GLUT-2 gene levels. In the liver tissue of diabetic and HFD rats, the translocation of GLUT-2 was decreased compared with the normal controls. This is quite normal when insulin is deficient in the diabetic state would decrease the translocation of GLUT-2 liver cell membranes. HEAA (500mg) treatment resulted in the reversal of GLUT-2 gene expression levels. There is evidence suggesting metformin's role in inhibiting glycogenesis especially in hepatic cells and improving insulin sensitivity. The translocation of GLUT2 to the apical membrane is promoted by metformin *via* the AMPK pathway thereby inhibiting glycogenesis [22].

PPARα, which plays an important role in lipids' metabolism, was also found to have significance in diabetes mellitus type 2. It helps in stimulating β cells to secrete insulin and protects the cells against damage [13]. The expression of PPARα was higher in the HEAA treatment groups compared to the control group. This indicates PPARα will help in protecting the β cells and in promoting the secretion of insulin.

The anti-diabetic and antioxidant activity of the herb *Achyranthes aspera* in diabetic-induced animal models has been established for a long time [23]. The same results were obtained when 500mg of *Achyranthes aspera* was administered.

Some studies analyzed the anti-diabetic and antioxidant activity of the herb *Achyranthes aspera* in diabetic-induced mouse models [23]. Another study also determined no significant toxicity changes and behavioural changes after the administration of *Achyranthes aspera* extract [5]. Previous results indicated that the roots of *Achyranthes aspera* exhibited anti-oxidant and antibacterial properties when compared with stem extracts [24]. From the results, it can be seen that *Achyranthes aspera* has both anti-diabetic and anti-hyperlipidemic properties. The insulin-mimetic effect of HEAA might add to glucose uptake through regulation in the expression of genes of the glucose transporter GLUT-2and PPAR-α in the liver.

CONCLUSION: This study showed the efficacy of *Achyranthes aspera* extract on managing not only diabetes mellitus but also the complications of high-fat diet through balancing antioxidants and also by regulating the mechanism of GLUT-2 and PPAR-α gene expression

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REFERENCES:

1. Brouns F: Overweight and diabetes prevention: is a low-carbohydrate–high-fat diet recommendable. European Journal of Nutrition 2018; 57(4): 1301–1312.
2. Newsholme P, Cruzat VF, Keane KN, Carlessi R & De Bittencourt PIH: Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochemical Journal 2016; 473(24): 4527–4550.
3. Wu Y, Ding Y, Tanaka Y & Zhang W: Risk Factors Contributing to Type 2 Diabetes and Recent Advances in the Treatment and Prevention 2014; 11(11): 1185.
4. Srivastav S, Singh P, Mishra G, Jha KK & Khosa RL: *Achyranthes aspera*-An important medicinal plant : A review. J Nat Prod Plant Resour 2011; 1(1): 1–14.
5. Kumar SV, Sankar P & Varatharajan R: Anti-inflammatory activity of roots of *Achyranthes aspera*. Pharmaceutical Biology 2009; 47(10): 973–975.
6. Dwivedi S, Dubey R & Mehta K: *Achyranthes aspera* Linn. (Chirchira): a Magic Herb in Folk Medicine. International Journal of Biomedical and Advance Research 2011; 2(6): 670–676.
7. Khan N, Akhtar MS, Khan BA, Braga VDA & Reich A: Antiobesity, hypolipidemic, antioxidant and hepatoprotective effects of *Achyranthes aspera* seed saponins in high cholesterol fed albino rats. Arch Med Sc 2014; 11(6): 1261-71.
8. Krishnakumari S and Priya K: Hypolipidemic efficacy of *Achyranthes aspera* on lipid profile in sesame oil fed rats. Ancient Science of Life 2006; 25(3-4): 49.
9. Saba Hasan: Pharmacological and Medicinal Uses of *Achyranthes aspera*. International Journal of Science, Environment and Technology 2014; 3(1): 123-129.
10. Otitoju O & Onwurah I: Superoxide Dismutase (Sod) Activity And Serum Calcium Level In Rats Exposed To A

Locally Produced Insecticide "Rambo Insect Powder." Animal Research International 2008; 2(1): 261–266.
11. Boriskin P, Deviatkin A, Nikitin A, Pavlova O & Toropovskiy A: Relationship of catalase activity distribution inserum and tissues of small experimental animals. IOP Conf Ser Earth Environ Sci 2019; 403: 12113.
12. Razygraev AV, Baziyan EV, Polyanskikh LS & Petrosyan MA: Experience of measuring glutathione peroxidase activity in surgically induced endometrial-like lesions in rats. Journal of Obstetrics and Women's Diseases 2021; 70(2): 55–61.
13. Devaki M, Nirupama R & Yajurvedi HN: Chronic stress-induced oxidative damage and hyperlipidemia are accompanied by atherosclerotic development in rats. The Inte J on the Biology of Stress 2013; 16(02): 233–243.
14. Leung PS & Chan YC: Role of Oxidative Stress in Pancreatic Inflammation 2009; 11(1): 135–165.
15. Piché ME, Tchernof A & Després JP: Obesity Phenotypes, Diabetes, and Cardiovascular Diseases. Circulation Research 2020; 1477–1500.
16. Armstrong JA, Cash N, Soares PMG, Souza MHLP, Sutton R & Criddle DN: Oxidative stress in acute pancreatitis: Lost in translation. Free Radical Research 2013; 47(11): 917–933.
17. Christensen AA & Gannon M: The Beta Cell in Type 2 Diabetes. Current Diabetes Reports 2019; 19(9).
18. Saisho Y, Butler AE, Meier JJ, Monchamp T, Allen-Auerbach M, Rizza RA & Butler PC: Pancreas volumes in humans from birth to age one hundred taking into account sex, obesity and presence of type-2 diabetes. Clinical Anatomy 2007; 20(8): 933–942.
19. Batista TM, Haider N & Kahn CR: Defining the underlying defect in insulin action in type 2 diabetes. Diabetologia 2021; 64(5): 994–1006.
20. Adeyi AO, Idowu BA, Mafiana CF, Oluwalana SA, Ajayi OL & Akinloye OA: Rat model of food-induced non-obese-type 2 diabetes mellitus: comparative pathophysiology and histopathology. International Journal of Physiology, Pathophysiology and Pharmacology 2012; 4(1): 51.
21. Ickin Gulen M, Guven Bagla A, Yavuz O & Hismiogullari A: Histopathological changes in rat pancreas and skeletal muscle associated with high fat diet induced insulin resistance. Biotechn and Histochem 2015; 90(7): 495–505.
22. Hsu S, Rain L and Franger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 1981; 29: 577-580.
23. Talukder FZ, Alam A, Khan KA, Uddin R, Jahan N & Alam A: *In-vitro* free radical scavenging and anti-hyperglycemic activities of *Achyranthes aspera* extract in alloxan-induced diabetic mice. Drug Discoveries & Therapeutics 2011; 6(6): 298–305.
24. Nivrutti Deshmukh V, Vasant Shewale V, Raghunath Narkhede M & Murlidhar Aurangabadkar V: *In-vitro* antioxidant activity of *Achyranthes aspera* L. Journal of Pharmacy Research 2009; 2(9): 1402–1403.

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