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ANTI-DIABETIC ACTIVITY OF *HOLORRHENA ANTIDYSENTRICA* (LINN) WALL, BARK ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The objective of the present study was to evaluate an antidiabetic potential of bark of *Holorrhena antidysentrica* (Linn). In the present study, the bark of *Holorrhena antidysentrica* (Linn) was screened for antidiabetic activity. The bark of *Holorrhena antidysentrica* (Linn) was subjected to hot continuous extraction (soxhlet) with ethanol as universal solvent, and fractionation was carried out to obtained pet.ether and chloroform fraction. Aqueous extract was prepared by cold maceration. After qualitative phytochemical investigations, all the extracts and fractions were subjected for antidiabetic activity in streptozotocin induced diabetes in rats. All the extracts and fractions were given orally at a dose of 300mg/kg body weight. Metformin was used as standard drug (250mg/kg body weight p.o.). The alcoholic as well as aqueous showed significant antidiabetic activity as compared to streptozotocin induced diabetes in rats.

Introduction –

It is well known that diabetes mellitus is the commonest endocrine disorder that, according to the World than 176 million people worldwide, in Mexico the WHO estimates that the Health Organization, affects more number of diabetic patients will increase from more than 2 million in 2002 to more than 6 million in 2030, which would imply that in a few decades Mexico may have highest rate of diabetes in the world. Because of the complications linked to diabetes like heart disease, retinopathy, kidney disease and neuropathy, it also is a common cause of chronic morbidity and disability among the working population. The term diabetes mellitus describes a metabolic disorder of multiple a etiologies and characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. The various extracts of the dried bark of *Holorrhena antidysentrica* is used traditionally for the treatment of diabetes.[2, 3]. Aqueous and ethanolic extracts of *Holorrhena antidysentrica* Wall seeds are significantly proved to possess anti-diabetic activity [4]. Fruit extract (50% ethanol) of *Holorrhena antidysentrica* was also showed hypoglycemic activity in rats [5]. The aim of present study was to investigate the hypoglycemic effect of aqueous, alcoholic extracts and fraction of pet. ether and chloroform, histopathological changes and tissue lipids in streptozotocin induced rats for *Holorrhena antidysentrica*.

Materials and methods –

Plant material

The bark of *Holorrhena antidysentrica* was collected from local areas of Sahyadri Hill ranges of Pune, identified and authenticated from Botanical Survey of India, Pune. A specimen voucher (No.SB01) of the same is deposited in Dept. of Pharmacognosy, Sharadchandra Pawar College of Pharmacy, Otur (Dumberwadi).

Extraction of plant material

The collected bark of *Holorrhena antidysentrica* was shade dried, powdered and extracted with ethanol as a solvent using a Soxhlet extractor (25 cycles) at 5 batches. After exhaustive extraction, the collected extract was dried under reduced pressure using rotary flash evaporator and dried at room temperature to obtain the residue. The part of extract was used for fractionation with pet ether and chloroform.

The aqueous extract was being obtained by subjecting the drug to the maceration process carried out for 7 days using the distilled water with occasional stirring and concentrating the supernant liquid to

the small volume under the reduced pressure and then evaporated to the dryness. The aqueous and alcoholic extracts, pet ether and chloroform fractions were subjected for further anti-diabetic studies.

Animals

Male Swiss albino mice weighing between 20-25gm and male albino wistar rats weighing 150-200 gm were used for this study. The animals were obtained from animal house, Raj Biotech, Pune. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{0}C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw diet. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (1197/08c/CPCSEA) and were in accordance with the guidelines of the CPCSEA.

Antidiabetic screening

Acute toxicity study

The oral acute toxicity was carried out as per OECD guidelines. 300mg/kg b.w of each extract and fractions were taken as effective dose for evaluation of antidiabetic activity.[6]

Preparation of dose

The alcoholic, aqueous extract and fractions of pet. ether and chloroform of *Holorrhena antidysentrica* 300mg/kg b.w. were formulated as suspension in distilled water using 1% tween 80 as suspending agent. Since 1% tween 80 has negligible effect on normal blood glucose level (BGL). The strength of the suspension was according to the dose administered and was expressed as weight of dried extract.

Preparation of standard drug

Metformin (250mg/kg b.w.) was used as the standard drug for evaluating the antidiabetic activity. The metformin was prepared by dissolving it in 0.9% normal saline solution. The metformin was taken from Micro Lab, Bangalore. The strength of the suspension was adjusted to 20mg/0.1ml b.w.

Induction of Diabetes:

Steptozotocin (60mg/kg) b.w. in 0.1M citrate buffer, pH 4.5 was injected intarperitonially to induce hyperglycemias.[7] The animals were considered diabetic when the blood glucose level (BGL) raised beyond 150mg/dl of blood and this condition was observed at the end of 72hr. after inducing streptozotocin. The animals were segregated into six groups of six rat each taking into consideration the diabetic BGL.

Animals

Male Swiss albino mice weighing between 20-25gm and male albino wistar rats weighing 150-200 gm were used for this study. The animals were obtained from animal house, Raj Biotech, Pune. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{0}C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw diet. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (1197/08c/CPCSEA) and were in accordance with the guidelines of the CPCSEA.

Methodology:

Before starting of the experiment, animals were separated according to their body weights. The animals were segregated into seven groups of six rats each, taking into consideration of diabetic blood sugar level. The alcoholic, aqueous and the fraction of pet. ether and chloroform of *Holorrhena antidysentrica* (300mg/kg b.w./oral) and the metformin (250mg/kg b.w./oral) and saline treated were orally administered, for every 24hrs. for a period of 7 days to rats using oral gauze.

Group I - control received normal saline.

Group II - received STZ 50mg/kg. b.w. by i.p

Group III- received STZ 50mg/kg. b.w. by i.p + metformin (250 mg/kg), p.o.

Group IV- received STZ 50mg/kg. b.w. by i.p + alcoholic extract of *Holorrhena antidysentrica* (300 mg/kg), p.o

Group V - received STZ 50mg/kg. b.w. by i.p + aqueous extract of *Holorrhena antidysentrica* (300 mg/kg), p.o

Group VI - received STZ 50mg/kg. b.w. by i.p + Chloroform fraction of *Holorrhena antidysentrica* (300 mg/kg), p.o

Group VII - received STZ 50mg/kg. b.w. by i.p + pet ether fraction of *Holorrhena antidysentrica* (300 mg/kg), p.o

The blood was collected in 1.5ml fluoride vial bottles. The blood glucose level was estimated in plasma on 1st, 3rd, 5th and 7th day of treatment. The OGTT was performed on the 7th day of the treatment. The blood samples were collected through retro-orbital puncture by micro hematocrit capillaries from fasted animals (12hrs.) under light ether anaesthesia.

Estimation of OGTT:

Estimation of the glucose for OGTT was carried out on the 7th day after treatment. The animals were fasted for 16hrs. After end of treatment, the dose of glucose (2gm/kg b.w.) was administered and the blood samples were collected at an interval of 0 hr., 1st hr., 3rd hr., 5th hr., and 7th hr. The blood sample was collected in fluorides tube and the plasma was separated and analyzed for the glucose (RFCL, Ltd.), cholesterol (RFCL, Ltd.), triglyceride (Span Diagnostic Ltd.) and HDL-cholesterol (RFCL, Ltd.) using diagnostic reagent kit.

Histopathological Evaluation:

Tissue samples from the pancreas were fixed in 10% buffered neutral formalin, embedded in paraffin screened at 5µm and stained with hematoxylin-eosin and periodic acid-schiff.

Statistical analysis-

The data's were expressed as mean ± SEM. Obtained from the number of experiment (n). One-Way ANOVA with Dunnet' post test was performed using Graph Pad Prism version 5.00 for windows.

Results-

Plasma Blood glucose

Administration of streptozotocin (60mg/kg) b.w. to the normal rats significantly elevated the blood glucose level compared with the rats injected the normal saline alone (table no.1) as in the previous reports. In the present study the extracts shows the significant hypoglycemic effects (table no.1). The aqueous and alcoholic extracts at the dose of 300 mg/kg b.w showed significantly reduced ($p<0.0001$) of plasma glucose level. When compared with streptozotocin induced rats. And Metformin (250 mg/kg b.w)

produced significant decrease in plasma glucose levels. The blood glucose level of untreated diabetic rats remains normal through out experimental period. The long administration of aqueous (300mg/kg b.w) has more significantly reduced blood glucose level from 1st day (190.1±26.14) to 7th day (99.54±6.23). Effect of blood glucose level in case of alcoholic extracts from 1st day (232.0±2.36) to 7th day (93.19±14.81). And similar effect were also shown for standard drug Metformin for the 1st day (171.0±18) and 7th day (86.01± 7.14). Thus the significant effect in reducing the blood glucose level was observed for alcoholic and aqueous extracts which were nearly equal to that of standard drug metformin. During an OGTT, the aqueous and alcoholic extract of *Holorrhena antidysentrica* significantly decreased the plasma glucose level throughout the study.

These findings clearly established the potency of aqueous and alcoholic extracts exhibits the anti-diabetic activity. As the aqueous and alcoholic extracts shows presence of alkaloids, steroids and tannins. As the literature revealed that alkaloids, steroids and tannins are known to reduce blood glucose level. [8]

Plasma Lipids

The effect of *Holorrhena antidysentrica* (300 mg/kg) on plasma lipids of normal and experimental rats is summarized in **table no. 3.** A marked increase in the frequency of cholesterol, triglyceride and hdl-cholesterol were observed in diabetic rats. Treatment with *Holorrhena antidysentrica* (300 mg/kg) significantly reduced the lipid levels.

Figure 1, 2 and figure 3 depicts the changes in blood glucose level, oral glucose tolerance test and fasting lipid profile respectively.

Histopathological observations

Figure A and figure B represent the islet of langerhans for normal and streptozotocin-induced diabetic rats respectively. Comparison of A and B clearly indicates the increase in the numbers of β-cells in diabetic rats. As it is evident that in figure B the islets are large are irregular shaped, most of cells of islets are small, degenerated and dark with scanty cytoplasm in all treated group of animals, severe vaculation and degeneration are present in the β-cells of numbers of islets.

However compared to streptozotocin induced diabetic rats histopathological examination of aqueous, alcoholic and chloroform fraction treated diabetic rats revealed bring to normal position. The presence of β-cells (figure C,D and E) and thus restoration of normal cellular population size of islets with hyperplasia by metformin (figure. F)

Discussion –

Streptozotocin is a well known for its selective pancreatic islets β cells toxicity and has been extensively used to induce the diabetes in an animals. It interferes with cellular metabolic oxidative mechanism. Intraperitonial administration of streptozotocin (60mg/kg b.w.) effectively induced diabetes in normal rats as reflected by hyperglycemia when compared with the normal rats. In our present investigations, we have observed that the aqueous and alcoholic extracts of *Holorrhena antidysentrica* bark can reverse this effect. The previous study showed that the excess of fatty acid in plasma produced by streptozotocin induced diabetes promotes the conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins. The abnormal high concentration of plasma lipids in the diabetes subject is due, mainly to increase in the

mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetes rats. And significant increase observed in our experiment was in accordance to these studies.[7] The plant extract treated diabetic samples histopathologically approach the corresponding healthy pancreatic samples. The regeneration of the β-cells of the STZ-destructed islets is probably due to the fact that pancreas contain stable cells which have the capacity of regeneration. Therefore, the surviving cells can proliferate to replace the lost cells. The renewal of β-cells in diabetes has been studied in several animal models. The total β-cells mass reflects the balance between the renewal and loss of these cells. [9]

It can be concluded from the data that aqueous and alcoholic extracts of *Holorrhena antidysentrica* reduces the level plasma glucose and plasma lipid level which are actively raised in streptozotocin diabetic rats.

The histopathological investigation along with the biochemical evaluation suggests the possibility of the islets regeneration upon plant extract treatment. Further research is required to explore exactly the mechanism of islet regeneration by the plant extract.

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Table 1. Effect of *H. antidysentrica* on plasma blood glucose level on different days (0day, 1st day, 3rd day, 5th day and 7th day) in normal and experimental animals:

Sr. No.	Group	Blood Glucose Level (mg/dl)				
		O day	**1st day**	**3rd day**	**5th day**	**7th day**
1	Normal	79.34± 3.705	79.80 ± 3.596	78.80 ± 3.42	80.34 ± 3.923	79.73 ± 3.473
2	STZ	248.5 ± 10.20***	224.7 ± 17.43***	232.4 ± 16.32***	229.5 ± 9.83***	204.2 ± 17.68***
3	Metformin	247.2 ± 12.93	171.0 ± 18.0	126.4 ± 1.32***	101.6 ± 6.69***	86.01 ± 7.14***
4	HA. Aq.	193.1 ± 12.82	169.6 ± 17.8	137.6 ± 3.703***	107.0 ± 6.489***	75.04 ± 6.327***
5	HA. Alc.	207.0 ± 10.27	215.0 ± 19.73	170.24 ± 24.04*	110.9 ± 5.678***	95.44 ± 10.76***
6	HA. Pet Ether	252.8 ± 14.76	240.5 ± 6.07	259.3 ± 9.84	220.7 ± 13.23	210.08 ± 22.94
7	HA. CHCL3	260.5 ± 3.64	224.5 ± 10.5	229.2 ± 13.76	237.2 ± 11.82	206.4 ± 10.96

Values are Means ± SEM for six rats in each group

One way ANOVA followed by Dunnett's test.

STZ is compared with normal by using unpaired t-test

*P<0.05, **P<0.01, ***P<0.001 compared with STZ

Table 2. Effect of *H. antidysentrica* and OGTT:

Sr. No.	Group	Blood Glucose Level (mg/dl) OGTT				
		O hr.	**1st hr.**	**3rd hr.**	**5th hr.**	**7th hr.**
1	Normal	79.73 ± 3.473	94.07 ± 2.787	87.86 ± 2.965	81.31 ± 2.785	79.69 ± 2.202
2	STZ	204.2 ± 17.68***	250.9 ± 10.23***	241.3 ± 8.399***	236.2 ± 6.73***	233.9 ± 6.860***
3	Metformin	86.01 ± 7.14***	135.5 ± 17.68***	98.29 ± 8.094***	89.88 ± 4.808***	80.39 ± 3.88***
4	HA. Aq.	75.04 ± 6.327***	160.9 ± 8.366***	119.3 ± 4.775***	95.34 ± 6.840***	92.01 ± 4.483***
5	HA. Alc.	95.44 ± 10.76***	158.0 ± 11.61***	118.6 ± 12.40***	121.65 ± 6.588***	103.1 ± 2.18***
6	HA. Pet Ether	210.08 ± 22.94	204.4 ± 4.772	171.6 ± 6.780***	238.5 ± 6.87	230.2 ± 7.45
7	HA. CHCL3	206.4 ± 10.96	214.9 ± 2.14	214.0 ± 8.516	180.4 ± 23.38**	221.7 ± 7.33

Values are Means ± SEM for six rats in each group

One way ANOVA followed by Dunnett's test.

STZ is compared with normal by using unpaired t-test

*P<0.05, **P<0.01, ***P<0.001 compared with STZ

Table No. 3 Effects of *H. antidysentrica* and changes in plasma levels of cholesterol, triglyceride and HDL-cholesterol in normal and experimental animal:

Sr. No.	Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-Cholesterol (mg/dl)
1	Normal	19.52 ± 0.6791	76.04 ± 1.473	26.60 ±2.542
2	STZ (60mg/kg b.w.)	65.08 ± 5.489***	123.0 ± 3.027***	19.13 ± 0.6223*
3	Metformin (250mg/kg b.w.)	28.34 ± 2.616***	79.75 ± 4.386***	39.39 ± 2.74***
4	Aq. HA (350mg/kg b.w.)	36.44 ± 2.677***	85.18 ± 9.325**	44.54 ± 3.277***
5	Alc. HA (350mg/kg b.w.)	45.60 ± 1.269***	102.8 ± 4.17	37.89 ± 1.260***
6	CHCL3 HA (350mg/kg b.w.)	70.49 ± 1.481	126.6 ± 7.231	24.00 ± 2.525
7	Pet. Ether HA (350mg/kg b.w.)	24.52 ± 0.9651***	68.93 ± 10.09***	24.24 ± 2.748

Values are Means ± SEM for six rats in each group

One way ANOVA followed by Dunnett's test.

STZ is compared with normal by using unpaired t-test

*P<0.05, **P<0.01, ***P<0.001 compared with STZ

Figure 1. Effect of *H. antidysentrica* on plasma blood glucose level on different days (0day, 1st day, 3rd day, 5th day and 7th day) in normal and experimental animals:

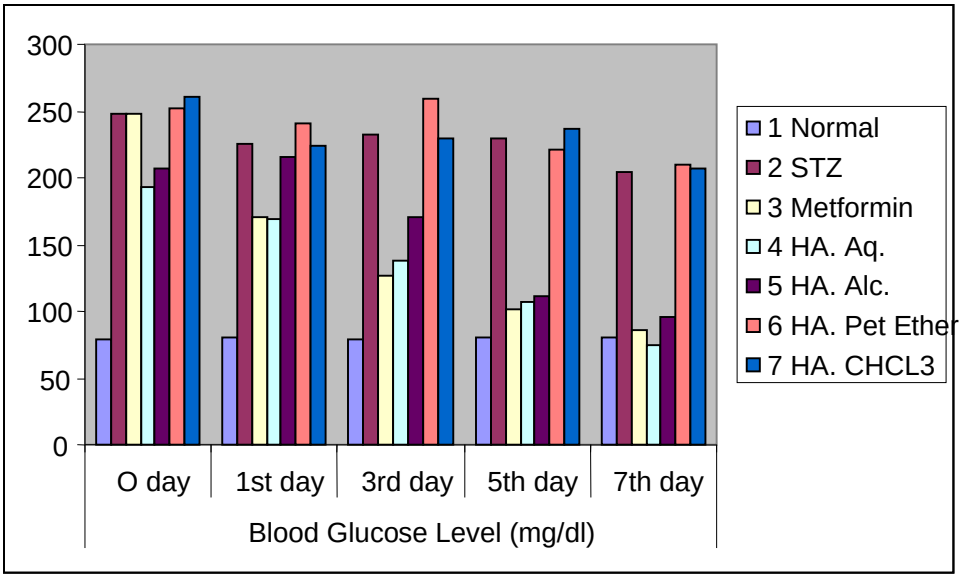


Figure 2. Effect of *H. antidysentrica* and oral glucose tolerance test (OGTT)

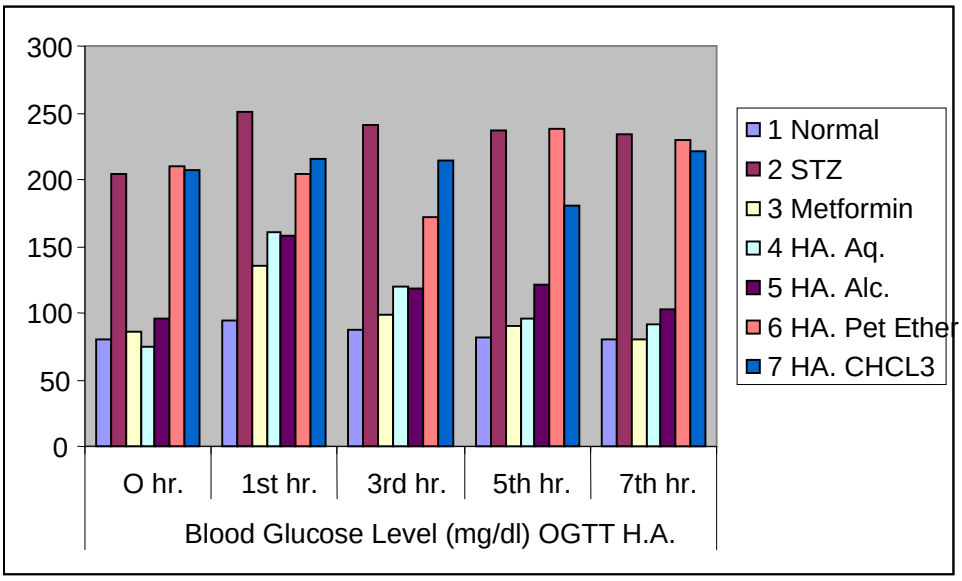
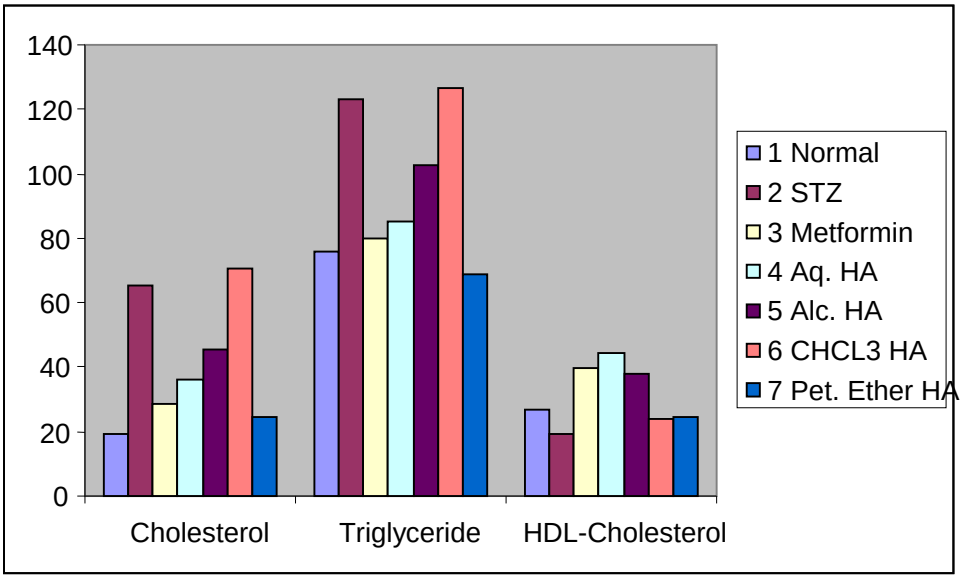


Figure 3. Effect of *H. antidysentrica* on fasting lipid profile:



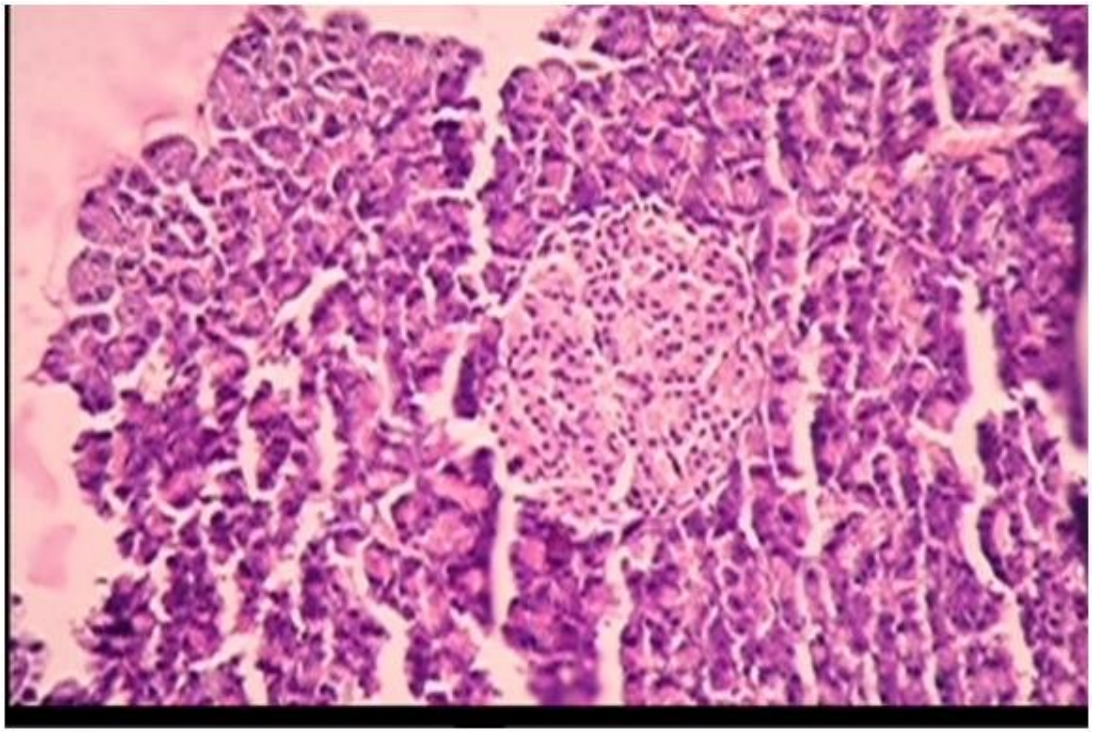


Figure A: Normal

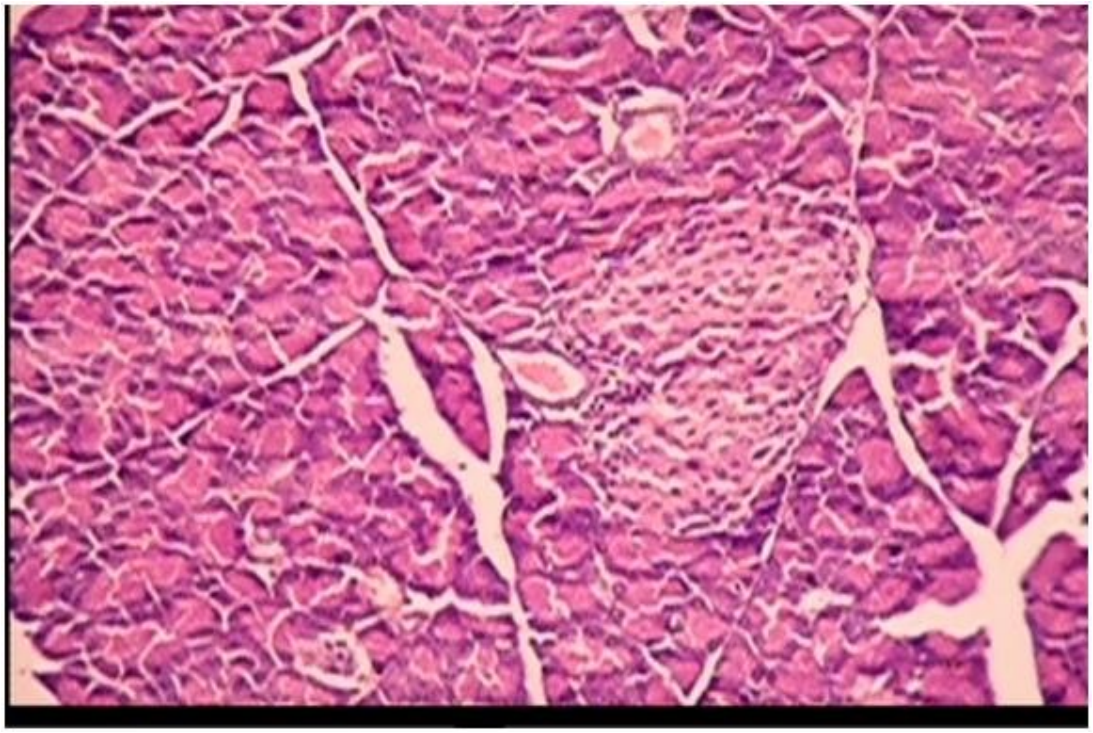


Figure B: STZ induced diabetic

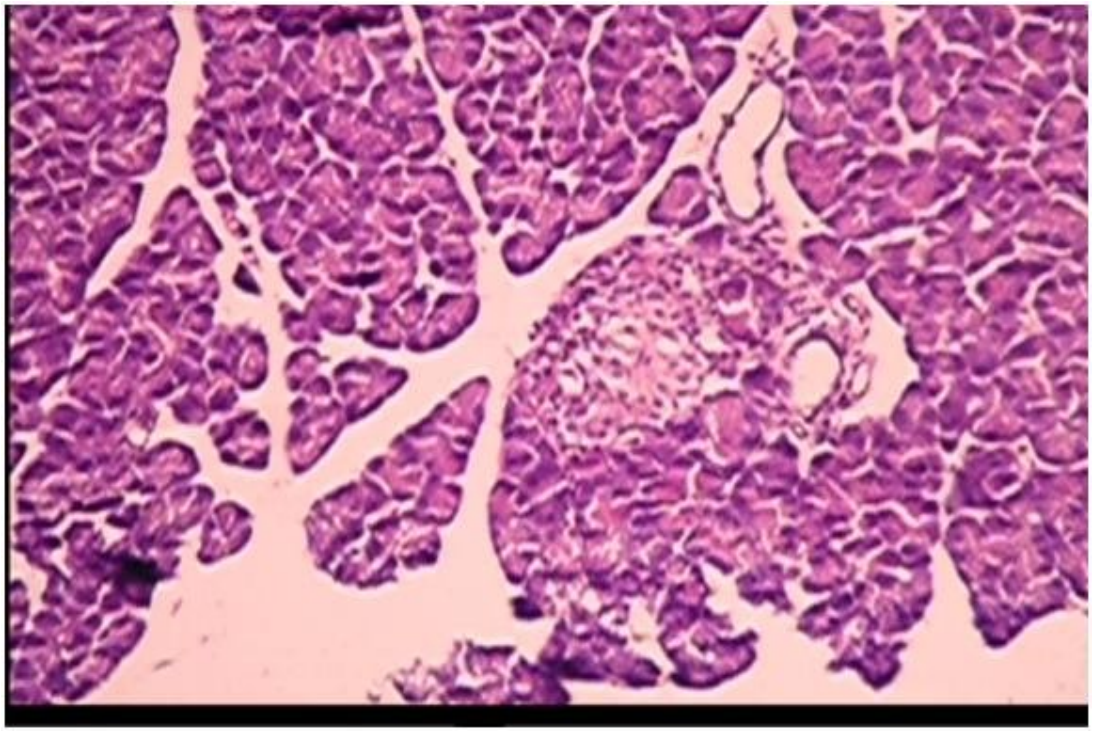


Figure C: Diabetic treated with 300 mg/kg bw aqueous extract

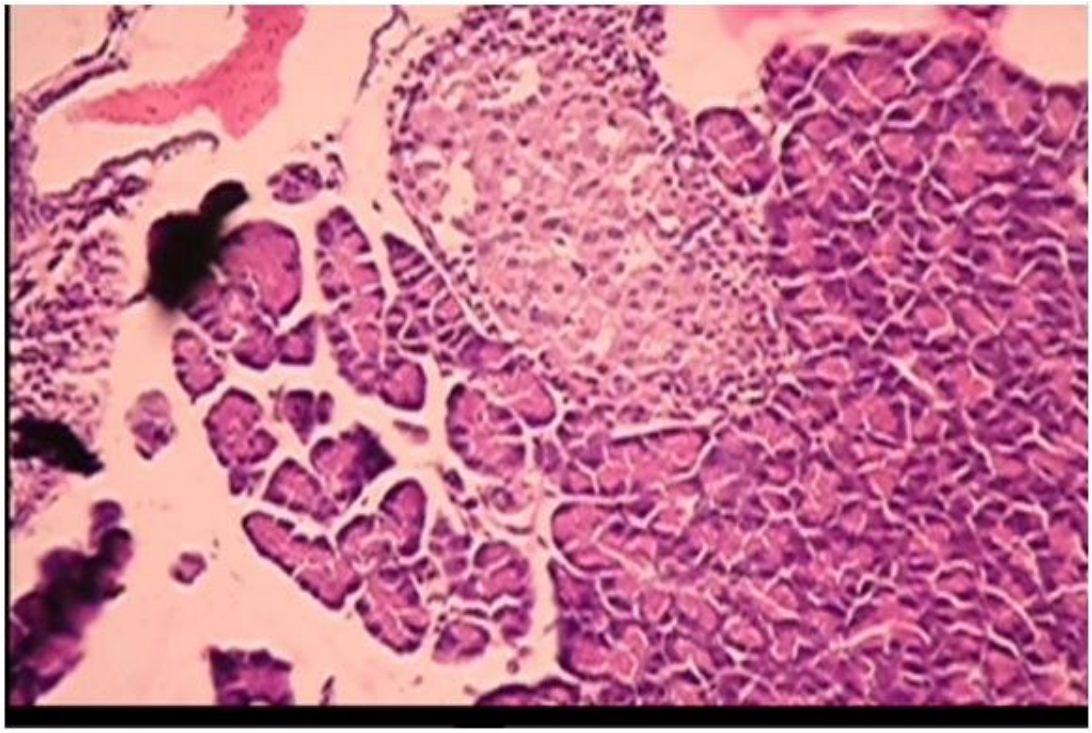


Figure D: Diabetic treated with 300 mg/kg bw alcoholic extract

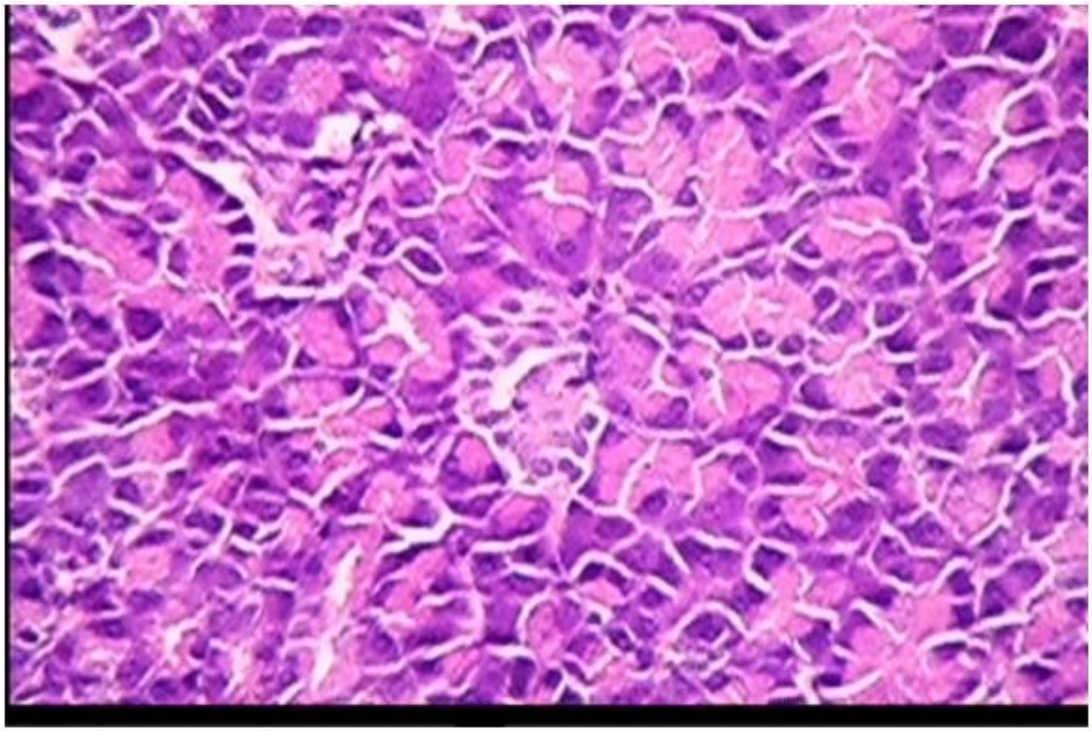


Figure E: Diabetic treated with 300 mg/kg bw chloroform fraction

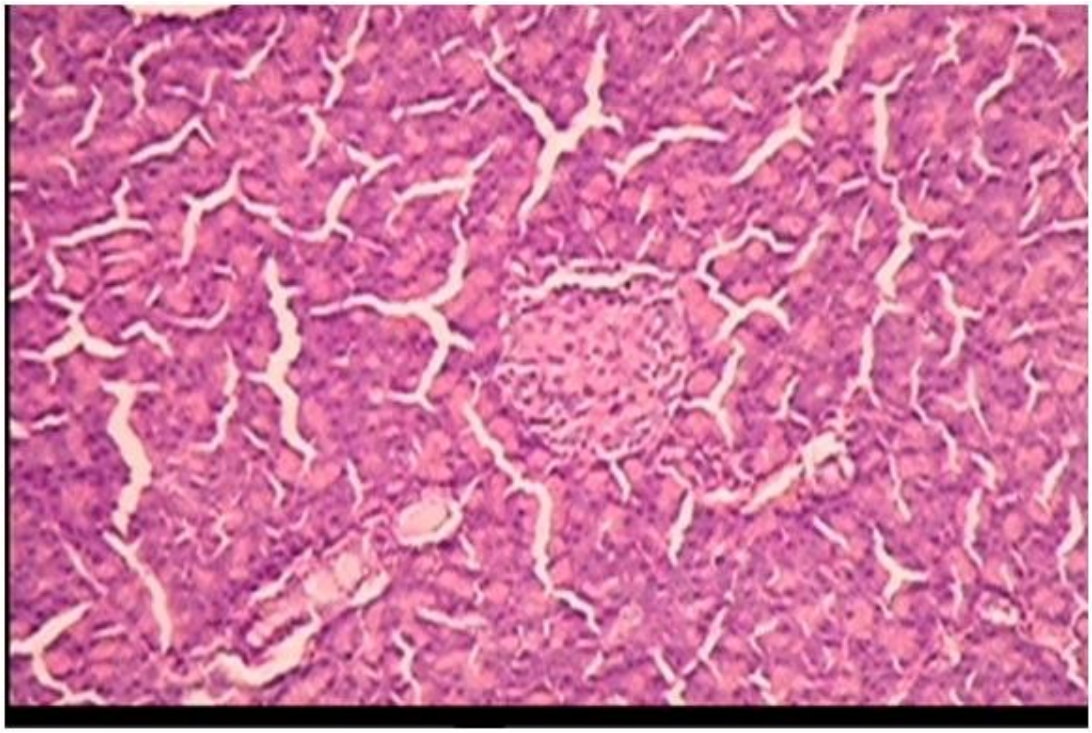


Figure F: Diabetic treated with 250 mg/kg b.w metformin

Legends for figures

Figure 1. Effect of *H. antidysentrica* on plasma blood glucose level on different days (0day, 1st day, 3rd day, 5th day and 7th day) in normal and experimental animals:

Figure 2. Effect of *H. antidysentrica* and oral glucose tolerance test (OGTT)

Figure 3. Effect of *H. antidysentrica* on fasting lipid profile:

Figure A: Normal

Figure B: STZ induced diabetic

Figure C: Diabetic treated with 300 mg/kg b.w aqueous extract

Figure D: Diabetic treated with 300 mg/kg b.w alcoholic extract

Figure E: Diabetic treated with 300 mg/kg b.w chloroform fraction

Figure F: Diabetic treated with 250 mg/kg b.w metformin