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ANTIDIABETIC ACTIVITY OF SEED EXTRACT OF *HOLOPTELEA INTEGRIFOLIA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Background: *The plant Holoptelea integrifolia (syn: Indian elm tree) therapeutically used as a leprosy, abdominal disorder, obesity, tumour, diarrhea, inflammation and diabetes.* ***Objective:*** *To investigate the therapeutic usefulness of ethanolic seed extract of Holoptelea integrifolia in the treatment of Diabetes mellitus.* ***Methods:*** *STZ was administered to rats (65 mg/kg i.p. per body wt.) to induce insulin deficient diabetes. They were treated with the seed of Holoptelea integrifolia ethanolic extract(250 and 500 mg/kg p.o.) daily from day 4 to 17 and fasting blood glucose, TG, Total Cholesterol, HDL, LDL levels were determined on day 17.* ***Result:*** *There is a significant increase ($p<0.001$) in blood glucose level, TG, Total cholesterol, VLDL, HDL and LDL respectively ($p<0.01$) in STZ control. Treatment with ethanolic seed extract of Holoptelea integrifolia at high dose (500 mg/kg B.W.) shown decrease ($p<0.001$) in blood glucose level, LDL, TG, Total cholesterol, VLDL, HDL and LDL ($P<0.01$).* ***Conclusion:*** *From results, it was concluded that Holoptelea integrifolia show anti diabetic activity in rats.*

KEY WORDS

Diabetes mellitus, Holoptelea integrifolia, Streptozotocin, Liver, Pancreas

Introduction:

Diabetes mellitus (DM), a leading metabolic disorder worldwide, characterized by hyperglycemia associated with impairment in insulin secretion and/or insulin action as well as alteration in intermediary metabolism of carbohydrate, protein and lipids (Alberti and Zimmet, 1998). Several reports indicate that annual incidence rate of diabetes mellitus will increase in future worldwide, especially in India. It has been proposed that approximately 57 million Indians will be affected by diabetes mellitus in the year 2025 (King et al., 1998; Kameswar rao et al., 2003). The World Health Organization (WHO) has suggested that over the next two decades, DM in the developing countries will be seen more in the lesser age group ranging from 20 to 45 years (Tierney et al., 2002). The WHO recognizes three main forms of diabetes mellitus: type 1, type 2, and gestational diabetes (occurring during pregnancy) (WHO, 1999).

Type-1 diabetes mellitus (IDDM or Juvenile-onset diabetes): In this type there is deficiency of insulin due to autoimmune destruction of β-cells. Destruction of β-cells may be due to viral infections, exposure to antigens. This increased level of glucose which causes metabolism to form acetoacetate & ketoglutarate. This leads to diabetic ketoacidosis. Treatment is done by giving insulin preparations.

Type-2 diabetes mellitus (NIDDM or Maturity-onset diabetes): In this type of DM there is secretion of insulin but due to insulin resistance & less secretion of insulin there is no action of insulin. This type of DM occurs in adults due to increase in age the β-cell function declines. This causes due to; decreased function of glucoreceptors on β-cells, decreased no. of insulin receptors reduced sensitivity a of peripheral tissues insulin receptors. Treatment is done by giving oral hypoglycemic.

Rationale of the work: The presence of steroids and glycosides and tannins in leaf extract showed

antidiabetic property. Presence of beta sitosterol, friedelin and epi friedelin in stem bark showed the antidiabetic property. Seed contains steroids, glycosides, tannins, beta sitosterol, friedelin and epi friedelin so it may contains antidiabetic property.

PLANT PROFILE: Holoptelea *integrifolia Holoptelea integrifolia* (Ulmaceae) commonly known as Indian Elm tree is a small to large deciduous tree that distributed throughout the greater part of India utan altitude of 2000 ft. The plant is being used by tribal people for their medicinal properties.

Chemical constitutes HolopteleaA and B, Friedelin, Friedelin-3-B-ol, B-sitosterol, Hederagenin, B-amyrin, Epifriedelinol, Hexacosanol, Octacosanol, Myristic, Stearic, Linoleic. The presence of phytochemicals in leaves is flavonoids, saponins, terpenoids, tannins, glycosides, steroids and anthraquinones. Therapeutic uses are Leprosy, Abdominal disorder, Obesity, diabetics.

MATERIALS AND METHODS:

Collection of Plant Materials: For the present investigation, the seeds of ***Holoptelea integrifolia*** were collected from different regions of hanamkonda after authentified by an expert taxonomist **M.D.Mustaffa,** Assistant professor, Department of botany, Kakatiya University, Warangal.

Preparation of Plant Extract The seeds were dried under shade and then powdered and stored in airtight container. The dried powder (240gm) material of the seeds was defatted with petroleum ether and the mare thus obtained was then extracted with ethanol (2110m1) in a soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained i.e. (12.5 % w/w). The dried extract was mass was obtained suspended in normal saline and used for the present study.

Experimental Animals: Male albino rats of Wister strain weighing 180-250g were procured from **Jeevan life sciences pvt.ltd,** Hyderabad, India. The animals were housed in a bio-safe temperature controlled environment with a 12:12 light/dark cycle with standard conditions of temperature (25±2^{0}C) and relative humidity (30-70%) during the experimental period. The animals were fed with standard pellet diet and water *ad libitum*. All the animals were acclimatized under laboratory conditions for a week before the commencement of experiments. The study was approved by the Institutional Animal Ethics Committee (IAEC), University college of Pharmaceutical Sciences, Hanamkonda, Warangal, India (**Protocol No: IAEC/7/UCPSC/KU/2014)**. The norms for Good Laboratory Practice (GCP) were followed for care of laboratory animals. The animals were maintained in accordance with the CPCSEA guidelines

EXPERIMENTAL DESIGN:

Male albino Wistar rats weighing between 180-250gms were used for the experiment and were allowed to acclimatize for a week. NO of groups=5. No of animals in each group=6.

Group I - Normal untreated rats.

Group II – Diabetic untreated rats with 65 mg/kg body weight of Streptozotocin.

Group III – Diabetic rats treated with 5mg/Kg body weight of Glibenclamide.

Group IV–Diabetic rats treated with 250mg/Kg body weight Holoptelea integrifolia seed extract.

Group V– Diabetic rats treated with 500mg/Kg body weight of Holoptelea integrifolia seed extract.

Animal models: Streptozotocin induced diabetic model in rats.

Drugs: Streptozotocin was prepared by dissolving 100mg in 5ml citrate buffer.

Glibenclamide was prepared by dissolving in acacia suspension

Extract was prepared by dissolving in acacia suspension.

Estimation time: After 17 days of the experiment blood samples were collected from retro orbital plexus for the biochemical estimation of glucose, triglycerides, total cholesterol, SGOT, SGPT, HDL and total protein. Then rats were sacrificed by ether anesthesia, pancreas was isolated for histopathological studies

Estimation of parameters and Results:

Estimation of glucose: Estimation of glucose by GOD/POD method (Trinder, 1969)

Estimation of SGPT: Estimation of SGPT by IFCC method (1986)

Estimation of SGOT: Estimation of SGOT by IFCC method (1986)

Estimation of total Cholesterol: Estimation of cholesterol by CHOD/PAP method (Trinder, 1969).

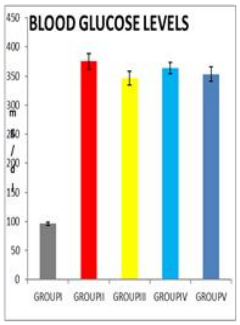
Estimation of Triglycerides: Estimation of Triglycerides by GPO/PAP method (Trinder, 1969)

Estimation of Total Proteins: Estimation of total proteins by biuret method (Gornet, 1949).

Estimation of HDL, VLDL and LDL: Serum HDL Cholesterol: (Trinder P., 1960)

Effect of seed extract of ***holoptelea integrefolia*** on blood glucose levels in (mg/dl) in STZ induced diabetic rats on day 0

S.No	Groups	mean± SD,
1	I-Vehicle control	96±.251
2	II-Diabetic control(STZ)(65mg/kg)	375±13.2#
3	III- STZ=Glibenclamide (5mg/kg)	346±11.77**
4	IV-STZ+H.I EXTRACT (250mg/kg)	364±9.6***
5	V-STZ+H.I EXTRACT (500mg/kg)	353±12.3**

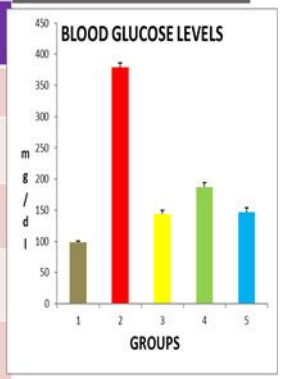


Blood glucose levels increases in all the diabetes induced groups compared to control.

Values are presented as mean ±SD, n= 6. #P< 0.001 compared with vehicle control group. ** p<0.05, *** p<0.001 compared with Positive control group. One way ANOVA followed by Tukey's multiple comparison tests

Effect of seed extract of ***holoptelea integrefolia*** on blood glucose levels in (mg/dl) in STZ induced diabetic rats on day 17

S.No	Groups	mean± SD,
1	I-Vehicle control	98±2.25
2	II-Diabetic control(STZ)(65mg/kg)	379±12.72#
3	III- STZ+ Glibenclamide (5mg/kg)	144±9.77b**
4	IV-STZ+H.I EXTRACT (250mg/kg)	187±4.27b***
5	V-STZ+H.I EXTRACT (500mg/kg)	147±12.91b**

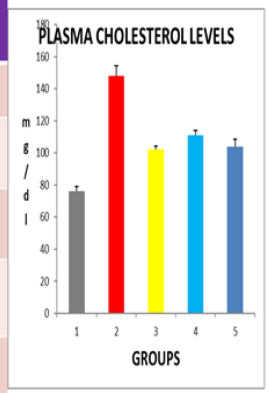


Blood glucose levels increases in all the diabetes induced groups compared to vehicle control group and decreased levels were found in treated rats

Values are presented as mean ±SD, n= 6. #P< 0.001 compared with vehicle control group. ** p<0.05, *** p<0.001 compared with Positive control group. One way ANOVA followed by Tukey's multiple comparison tests

Effect of seed extract of ***holoptelea integrefolia*** on plasma cholesterol(mg/dl) in STZ induced diabetic rats.

S.No	Groups	mean± SD,
1	I-Vehicle control	76.±3.173
2	II-Diabetic control(STZ)(65mg/kg)	148.5±11.96#
3	III- STZ+ Glibenclamide (5mg/kg)	102.4±1.78***
4	IV-STZ+H.I EXTRACT (250mg/kg)	117±2,296**
5	V-STZ+H.I EXTRACT (500mg/kg)	104.3±4.32***

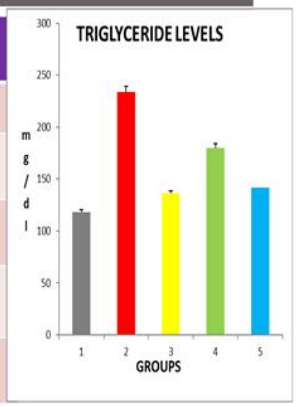


Plasma cholesterol levels were increases in all the diabetes induced groups compared to vehicle control group and decreased levels were found in treated rats

Values are presented as mean ±SD, n= 6. #P< 0.001 compared with vehicle control group. ** p<0.05, *** p<0.001 compared with Positive control group. One way ANOVA followed by Tukey's multiple comparison tests

Effect of seed extract of ***holoptelea integrefolia*** on plasma triglycerides levels (mg/dl) in STZ induced diabetic rats.

S.No	Groups	mean± SD,
1	I-Vehicle control	118.2±2.65
2	II-Diabetic control(STZ)(65mg/kg)	234.3±8.32#
3	III- STZ+ Glibenclamide (5mg/kg)	136.6±2.281**
4	IV-STZ+H.I EXTRACT (250mg/kg)	180.7±4.2***
5	V-STZ+H.I EXTRACT (500mg/kg)	142±4.42**

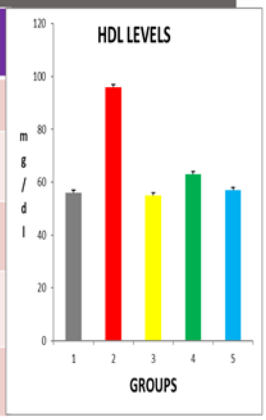


Triglyceride levels were increases in all the diabetes induced groups compared to vehicle control group and decreased levels were found in treated rats

Values are presented as mean ±SD, n= 6. #P< 0.001 compared with vehicle control group. ** p<0.05, *** p<0.001 compared with Positive control group. One way ANOVA followed by Tukey's multiple comparison tests

Effect of seed extract of ***holoptelea integrefolia*** on plasma HDL levels (mg/dl) in STZ induced diabetic rats.

S.No	Groups	mean± SD,
1	I-Vehicle control	58.2±1.8
2	II-Diabetic control(STZ)(65mg/kg)	96.6±3.36#
3	III- STZ+ Glibenclamide (5mg/kg)	55.33±0.96**
4	IV-STZ+H.I EXTRACT (250mg/kg)	63.5±4.42***
5	V-STZ+H.I EXTRACT (500mg/kg)	57.3±2.46**

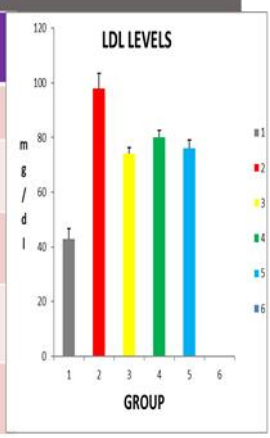


HDL levels increases in all the diabetes induced groups compared to vehicle control group and decreased levels were found in treated rats.

Values are presented as mean ±SD, n= 6. #P< 0.001 compared with vehicle control group. ** p<0.05, *** p<0.001 compared with Positive control group. One way ANOVA followed by Tukey's multiple comparison tests

Effect of seed extract of ***holoptelea integrefolia*** on plasma LDL levels (mg/dl) in STZ induced diabetic rats.

S.No	Groups	mean± SD,
1	I-Vehicle control	43.6±3.72
2	II-Diabetic control(STZ)(65mg/kg)	98.4±11.96#
3	III- STZ+ Glibenclamide (5mg/kg)	74.6±3.78**
4	IV-STZ+H.I EXTRACT (250mg/kg)	80.6±11.3**
5	V-STZ+H.I EXTRACT (500mg/kg)	76.3±4.32***

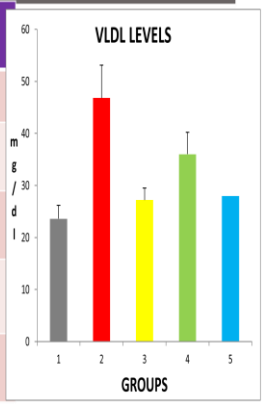


LDL levels increases in all the diabetes induced groups compared to vehicle control group and decreased levels were found in treated rats

Values are presented as mean ±SD, n= 6. #P< 0.001 compared with vehicle control group. ** p<0.05, *** p<0.001 compared with Positive control group. One way ANOVA followed by Tukey's multiple comparison tests

Effect of seed extract of ***holoptelea integrefolia*** on PLASMA VLDL levels (mg/dl) in STZ induced diabetic rats.

S.No	Groups	mean± SD,
1	I-Vehicle control	23.6±2.65
2	II-Diabetic control(STZ)(65mg/kg)	46.8±8.34#
3	III- STZ+ Glibenclamide (5mg/kg)	27.23±6.42***
4	IV-STZ+H.I EXTRACT (250mg/kg)	36.2±8.43***
5	V-STZ+H.I EXTRACT (500mg/kg)	28.4±4.32***

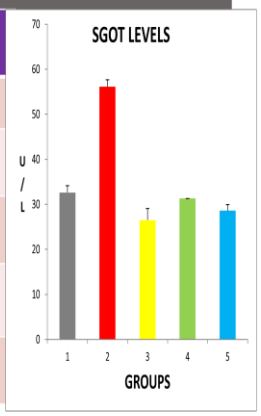


VLDL levels increases in all the diabetes induced groups compared to vehicle control group and decreased levels were found in treated rats

Values are presented as mean ±SD, n= 6. #P< 0.001 compared with vehicle control group. ** p<0.05, *** p<0.001 compared with Positive control group. One way ANOVA followed by Tukey's multiple comparison tests

Effect of seed extract of ***holoptelea integrefolia*** on SGOT levels (U/L) in STZ induced diabetic rats.

S.No	Groups	mean± SD,
1	I-Vehicle control	32.66±1.52
2	II-Diabetic control(STZ)(65mg/kg)	56.16±2.34#
3	III- STZ+ Glibenclamide (5mg/kg)	26.53±4.65**
4	IV-STZ+H.I EXTRACT (250mg/kg)	31.5±6.24***
5	V-STZ+H.I EXTRACT (500mg/kg)	28.5±5.24**

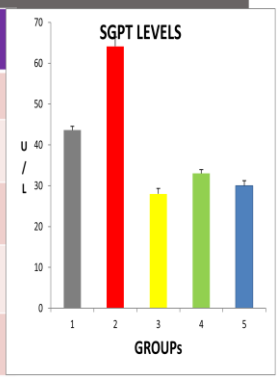


SGOT levels increases in all the diabetes induced groups compared to vehicle control group and decreased levels were found in treated rats

Values are presented as mean ±SD, n= 6. #P< 0.001 compared with vehicle control group. ** p<0.05, *** p<0.001 compared with Positive control group. One way ANOVA followed by Tukey's multiple comparison tests

Effect of seed extract of ***holoptelea integrefolia*** on SGPT levels (U/L) in STZ induced diabetic rats.

S.No	Groups	mean± SD,
1	I-Vehicle control	34.56±0.95
2	II-Diabetic control(STZ)(65mg/kg)	64.16±3.26#
3	III- STZ+ Glibenclamide (5mg/kg)	28.7±1.38***
4	IV-STZ+H.I EXTRACT (250mg/kg)	33.6±33.6**
5	V-STZ+H.I EXTRACT (500mg/kg)	30.1±2.13**

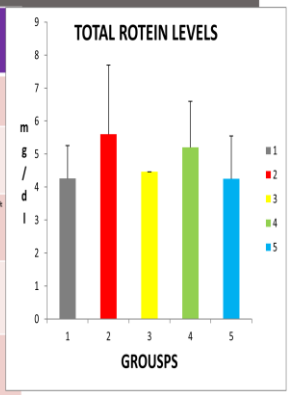


SGPT levels increases in all the diabetes groups compared to vehicle control group and decreased levels were found in treated rats..

. Values are presented as mean ±SD, n= 6. #P< 0.001 compared with vehicle control group. ** p<0.05, *** p<0.001 compared with Positive control group. One way ANOVA followed by Tukey's multiple comparison tests

Effect of seed extract of ***holoptelea integrefolia*** on total protein levels (mg/dl) in STZ induced diabetic rats.

S.No	Groups	mean± SD,
1	I-Vehicle control	4.26±2.03
2	II-Diabetic control(STZ)(65mg/kg)	5.46±3.24#
3	III- STZ+ Glibenclamide (5mg/kg)	4.46±0.523***
4	IV-STZ+H.I EXTRACT (250mg/kg)	5.21±1.54**
5	V-STZ+H.I EXTRACT (500mg/kg)	4.27±2.34***

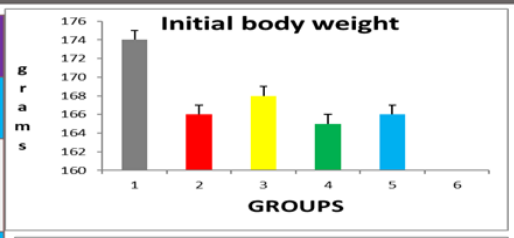


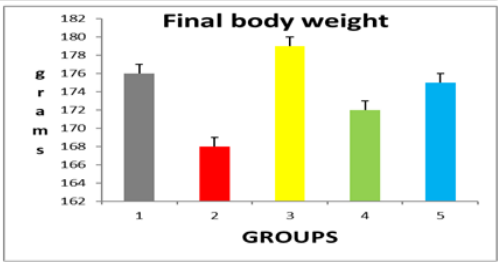
Total protein levels were increases in all the diabetes induced groups compared to vehicle control group and decreased were found in treated rats.

Values are presented as mean ±SD, n= 6. #P< 0.001 compared with vehicle control group. ** p<0.05, *** p<0.001 compared with Positive control group. One way ANOVA followed by Tukey's multiple comparison tests

Effect of seed extract of holoptelea integrefolia on body weight (grams) in STZ induced diabetic rats.

Groups	Initial body weight	Final body weight
I –Vehicle control	174.2±5.42	176±4.32
II-Diabetic control STZ(60mg/kg)	166.4±4.77	168±3.42
III-STZ+Glibenclamide(5mg/kg)	168.3±5.37	179.3±4.42
IV-STZ+H.I (250mg/kg)	165.7±4.32	172.5±3.42
V –STZ+H.I (500mg/kg)	166±5.32	175.3±5.67





The untreated diabetic rats gained weight at much lower rate compared to control and Holoptelea integrefolia extract treated rats. Administration of Holoptelea integrefolie extract diabetic groups IV V rats resulted in an increase in body weight compared to diabetic rats group.

Discussion:

The present research discussed about the antidiabetogenic effect of ethanolic extract of seeds of *Holoptelea Integrifolia* in streptozotocin-induced diabetic rat in dose dependent fashion. Streptozotocin injection resulted in idiabetes mellitus, which may be due to destruction of beta cells of Islets of Langerhans as proposed by others (Grover et al., 2000). Fasting blood glucose levels of untreated diabetic rats were significantly higher than in normal rats. Overproduction of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental bases of Hyperglycemia in diabetes mellitus (Latner, 1958). Diabetes induction caused significant ($P<0.001$) hyperglycemia (Table 2). Oral administration of the extract and glibenclamide for 14 days significantly ($P<0.001$) lowered the hyperglycemia of the experimental groups. The fasting blood glucose of the group treated with 500mg/kg body weight extract lowered the glucose level from 379.2 mg/dl to 147 mg/dl and glibenclamide from 370 mg/al to 144 mg/dl representing 78.15% and 77.83% reduction respectively. The effect on the fasting blood glucose is dose dependent. Among the two doses extract of 500 mg/kg dose had showed significant anti-hyperglycemic effect. As it is evident from the results that maximum reduction in the blood glucose levels were observed at 14^{th} day of treatment. Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites (Rej, 1978). SGOT and SGPT are reliable markers of liver function. Liver was necrotized in STZ-induced diabetic rats (Ohaeri, 2001). Therefore, an increase in the activities of SGOT and SGPT in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into blood stream (Navarro et al., 1993) which gives an induction of the hepatotoxic effect of STZ. Treatment of the diabetic rats with Glibenclamide (5mg/kg) and extract (250mg/kg) and (500mg/kg) caused reduction in the activity of these enzymes in plasma compared to the diabetic untreated group and consequently alleviated liver damage caused by STZ-induced diabetes. Lipid profile, which is altered in the stream of STZ-induced diabetic rats, appears to be a vital factor in the development of atherosclerosis which is noted in diabetes (Chattopadhyay and Bandyopadhyay, 2005). Elevated levels in serum TG and TC in diabetes are consistent with our previous observation (Maiti et al., 2005) and by others. In this study ethnolic extract significantly

recovered the levels of serum lipid profile in treated diabetic rats when compared to untreated diabetic rats. From this result, it may be stated that the ethanolic extract leads to regeneration of the beta cells of the pancreas and potentiating of insulin secretion from surviving beta cells. The increase in insulin secretion and consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. In this study STZ diabetic rats showed a significant increase in the serum total protein levels. Ethanolic extract significantly lowers the levels of serum total protein in treated diabetic rats when compared to untreated diabetic rats (Table 8) Diabetes induction significantly raised the levels of total cholesterol, total protein and triglycerides. The untreated diabetic rats gained weight at a lower rate compared to control and HI extract treated groups. Administration of HI to diabetic (Group IV & V) rats resulted in an increase in body weight compared to diabetic rats (Groups II). Results suggested that HI treatment has positive effect on maintaining body weights in diabetic rats. A gradual increase in body weights of Glibenclamide treated groups (Groups III) was similar to that of normal control rats. STZ induced diabetes (Baynes and Thorpe, 1999).

Similar studies on anti-hyperglycemic effect of aqueous and cold extracts of leaves of *Terminalia catappa,* ethanolic extract of powdered bark of *Diospyros melanoxylon* and *Vinca rosea* extract was reported and the proposed and the proposed mechanism was by regeneration of islet beta cells following destruction by alloxan (Syed mansoor ahmed et al., 2005; Ghosh and Suryawanshi 2001; Jadhav et al., 2009).

Histopathology of pancreas

Preparation of neutral formalin solution: Buffered neutral formalin solution is the best over fixative, therefore strongly recommended for routine use its contents are as follows. 37-40%formalin: 100ml Distilled water: 900ml. Sodium phosphate monobasic: 4.0grams. Sodium phosphate dibasic: 6.5grams

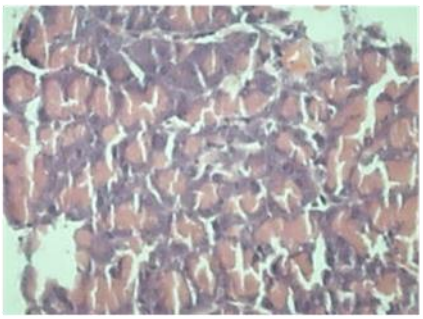
Histology of pancreas was observed for all groups. At the end of study rats of this group were scarified. Pancreas was isolated and fixed in buffered neutral formalin solution dehydrated with ethyl alcohol and then induced in paraffin. Sections of 5 micrometer were observed by microtone. Hematoxylin-eosin stain was applied to observe the histology pattern of pancreatic langerhans. Medical pathologist gave comment on histological observation.

Conclusion:

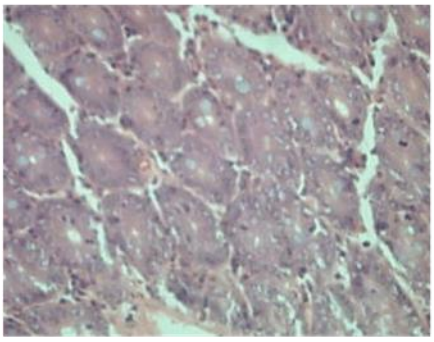
The current study indicates that ethanolic seed extract of Holoptelea Integrifolia produces significantly hypoglycemic activity in streptozotocin induced diabetes in rats. The mechanism of action may be due to its ability to modify insulin action. Ethanolic seed extract of *Holoptelea integrifolia* also reduce triglyceride , total cholesterol , LDL , VLDL level and increased HDL level significantly which show prevention and /or management of diabetes and the pre diabetic state of insulin resistance .

Further comprehensive biochemical and pharmacological investigations are needed to elucidate the exact mechanism of action of ethanolic seed extract of *Holoptelea integrifolia* and will be helpful in projecting this plant as therapeutic target.

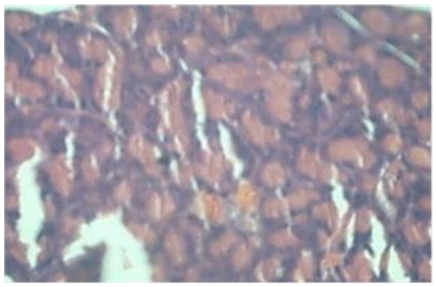
.A. Photograph of rat pancreas of the Normal control group administered distilled water(seen under 20x).



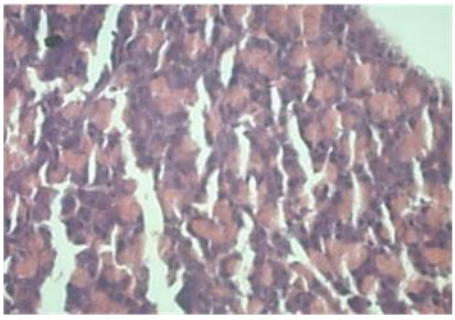
B. Photograph of rat pancreas of the STZ treated group administered 60mg/kg showing destruction of beta cells (seen under 20x).



C. Photograph of rat pancreas of the Glibenclamide treated group administered 5mg/kg showing decreased of beta cell destruction compared with STZ treated group (seen under 20x).



D. Photograph of rat pancreas of the Plant extract treated group administered 250mg/kg showing decrease of beta cell destruction compared with STZ treated group (seen under 20x).



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