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IN -VIVO EVALUATION OF ABIES PINDROW AS AN ANTI-DIABETIC PLANT

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Keywords:

Abies pindrow, Alloxan, Diabetes mellitus, glibenclamide

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ABSTRACT: Background: The present study was carried to evaluate the phytochemical, antioxidant, and anti-diabetic potential of *Abies pindrow* leaf extracts. **Methods:** The extracted leaf samples of *Abies pindrow* in methanolic and hydroalcoholic solutions were evaluated for phytochemical and antioxidant activities. *In-vitro* antioxidant activity of both test samples was calculated by DPPH radical scavenging capacity, reducing power activity, total phenolic and flavonoid contents. Further the study was carried to evaluate the anti-diabetic potential of hydroalcoholic extract of *Abies pindrow* leaves in Alloxan-induced diabetic rats for 21 days. Various biochemical parameters and histopathological studies were also carried out. **Results:** The hydroalcoholic extract of *Abies pindrow* was found to have more antioxidant potential than the methanolic extract. Also the same extract at high dose (400mg/kg) exhibited significant anti-diabetic activity than the low dose (200mg/kg) in alloxan-induced diabetic rats. The hydroalcoholic extract also showed improvement in parameters like body weight and lipid profile as well as regeneration of β-cells of the pancreas in alloxan-induced diabetic rats. Histopathological studies show reinforcement of the healing of pancreas by hydroalcoholic extract of *Abies pindrow* leaves, as a possible mechanism of their anti-diabetic activity. **Conclusion:** The results concluded that the plant extract was capable of managing diabetes and complications of diabetes. Hence may be considered as of the potential sources for the isolation of new oral anti-diabetic agents.

INTRODUCTION: Plants are the oldest medicine known to mankind. The ethnobotanical information reports 800 plants that may possess anti-diabetic potential [1]. Medicinal foods are prescribed widely even when their biologically active compounds are unknown because of their safety, effectiveness, and availability [2].

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The World Health Organization (WHO) has recommended evaluating traditional plant treatments for diabetes as they are effective, non-toxic, and have less or no side effects. The herbal anti-diabetic drug mainly belongs to plants, marine algae, and fungi to phytogenetically advanced classes of compounds.

Diabetes Mellitus and its complications cause numerous heath problems throughout the world[3]. It is becoming the third "killer" of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases [4]. The prevalence of diabetes Mellitus is expected to reach up to 4.4% in 2030 and the occurrence was found to be high in

India, China and USA. The prevalence of diabetes Mellitus is showing a rising trend in Kashmir valley because of increasing stress in the valley due to turmoil and changes in lifestyle [5, 6]. A cross-sectional population survey was done in the Kashmir valley in 2006, and the prevalence of 'known diabetes among adults aged >40 years was found to be 1.9% [7, 8.] Diabetes is a metabolic disorder that is a major cause of high economic loss. Medicinal plants, since immemorial times, have been used in virtually all civilizations as a source of medicine [9].

Metformin, less toxic biguanides, and the potent oral glucose-lowering agent were developed from *Galega officianalis* and used to treat Diabetes [7]. Out of dozens of oral medications for diabetes, only one medication (Metformin) is approved for use in children and originated from a herb. Medicinal plants that are the most effective and the most commonly studied about diabetes and its complications are *Gentiana olivieri, Bauhinia forficata, Eugenia jambolana, Lactuca indica, Mucuna pruriens, Tinospora cordifolia, Momordica charantia, Aporosa lindleyana, Myrtus communis and Terminalia pallid* [10]. Most plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., which are frequently implicated in anti-diabetic effects. Also, herbal formulations are preferred as they are safe and cheap when compared to synthetic oral hypoglycaemic drugs [11, 12].

Abies pindrow (Family: Pinaceae), commonly known as "West Himalayan Fir and Budal in Kashmiri is found mainly in Himalayas deciduous forests from Afghanistan to Nepal and all over the Western Himalayas [13, 14] . Trees are up to 30 m tall or more, with a narrow pyramidal shape, bark fissured, light grey to brown. Leaves are spiral needle-shaped, 1-4 cm long, upper surface grooved, dark green, and shiny. Traditionally, the plant has been used as an expectorant, anticatarrhal, antiseptic, antispasmodic, and carminative [15, 16]. The powder of leaves is used as a remedy for fever, and juice is employed as a tonic in parturition [17, 18]. Different studies have evaluated *Abies pindrow* for the presence of antioxidant, anti-ulcerant, analgesic, anti-inflammatory, hypotensive and anxiolytic activities [19, 20]. *Abies pindrow* has been claimed to possess anti-diabetic activity in traditional systems of medicine, but no systematic and scientific data are available to prove this activity [21, 22, 23]. Since Abies pindrow is found abundantly in Kashmir [24, 25], the present research work entitled "Anti-diabetic Activity of *Abies pindrow* Extract in Alloxan induced Diabetic Rats" using alloxan as the agent for inducing diabetes was conducted in experimental animals to scientifically validate their anti-diabetic activity [26, 27]. The *Abies pindrow* Extract was also evaluated for its phytochemical and *in-vitro* antioxidative potential [28, 29].

MATERIALS AND METHODS:

Identification and Collection: The leaves of *Abies pindrow* (AP); (Family: Pinaceae) was collected from Tangmarg, Kashmir. The identification of leaves was done by a taxonomist (Akhtar H. Malik). A sample of selected plant leaf was deposited in the herbarium of the Department of Taxonomy, the University of Kashmir, under voucher specimen number-*Abies pindrow*-2708-(KASH) for future reference. The evergreen leaves of the collected plant were dried in a hot house (18 to 37 °C) of the Centre for biodiversity and taxonomy, University of Kashmir.

Preparation of Methanolic Extract: The dried leaves of *Abies pindrow* were finely powdered, and 500gm was allowed to macerate for 48 h with methanol with occasional shaking. After 48 h, the methanolic extract was filtered through Whatman's filter paper. After filtration, the extract was evaporated to dryness with the help of rotavapour. The process was repeated with 250gm two times, and the total yield was noted.

Preparation of Hydroalcoholic Extract: The dried leaves of *Abies pindrow* were finely powdered, and 600gm was allowed to macerate for 48hrs with methanol and water in a ratio of 1:1 with occasional shaking. After 4 hrs the hydroalcoholic extract was filtered through Whatmans filter paper. After filtration, the extract was evaporated to dryness with the help of rotavapour. The process was repeated with 300gm two times, and the total yield was noted.

Phytochemical Analysis [30, 31]: The stoke solutions of methanolic extract (40mg extract in 40ml methanol) and hydroalcoholic extract (40mg

extract dissolved in 20ml methanol +20ml water) were respectively subjected to phytochemical analysis. The standard qualitative methods analysed the presence of various phytoconstituents. .

Evaluation of Antioxidant Potential [32, 33]:

- DPPH radical scavenging capacity.
- Reducing Power.
- Determination of total flavonoid content.
- Determination of total phenolic content.

Procurement, Approval and Exposure conditions of Wistar Rats: The wistar rats were obtained from the Indian Institute of Integrative Medicine, Canal Road Jammu. The animals were housed in well polypropylene cages in the animal house of the Department of Zoology, University of Kashmir. Male and female rats were placed in separate cages and provided with proper animal pellet feed and fresh water. They were kept at an ambient room temperature of 25°C ± 2°C and relative humidity 45-55% with 12 h light/12 h dark cycle. The undertaking of animals was performed according to CPCSEA guidelines after proper approval from the Institutional Animal and Ethics Committee (IAEC) at the Department of Regional Research Institute of Unani Medicine, Srinagar Reg. no. 927/GO/Re/S//CPCSEA-(Unani-Medicine).

Pharmacological study [34, 35]:

Acute Oral Toxicity Study: Extracts of *Abies pindrow* (AP) were administered orally in the range of 100mg-2000mg/kg and mortality was observed for 72hrs in various pharmacological and toxicity studies. Therefore, used safely in the current Anti-diabetic study.

***In-vivo* Antidiabetic Assessment of Hydroalcoholic Extract of *Abies pindrow* (AP) Leaves in Alloxan Induced Diabetic Rats [36, 37]:** In present study, hydroalcoholic extract of *Abies pindrow (*AP) (200 and 400mg/kg b.wt) were evaluated for anti-diabetic activity against Alloxan induced diabetes in rats. Rats were divided into 5 groups consisting of 6 rats in each group. After randomization into various groups and before the experiment, the rats were acclimatized for 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours *ad libitum*. The animals in each group receive the following treatment schedule:

Group I: Normal control (saline).

Group II: Alloxan treated control (150mg/kg,i.p).

Group III: Alloxan (150mg/kg,i.p) + AP extract (200mg/kg, p.o).

Group IV: Alloxan (150mg/kg,i.p) + AP extract (400mg/kg, p.o).

Group V: Alloxan (150mg/kg,i.p) + Standard drug, Glibenclamide (5mg/kg, p.o).

Abies pindrow (AP) extract and standard drug glibenclamide was dissolved in normal saline. Group, I served as the normal control group, which received normal saline (NS) for 21 days. AP extract, Standard drug and NS were administered orally (p.o) with a feeding cannula.

Group II to Group V was made diabetic control rats, and administered alloxan (i.p.).Group III, Group IV and Group V which previously received Alloxan were treated with AP extract (200mg/kg,p.o); (400mg/kg,p.o) and standard drug Glibenclamide (5mg/kg,p.o) respectively for 21 consecutive days.

Induction of Diabetes in Rats: After overnight fasting, rats were made diabetic by a single intraperitoneal injection of Alloxan monohydrate (150mg/kg b.w) prepared in sterile saline to all the groups except group I which served as normal control. Alloxan was first weighed individually for each rat according to their body weight and then solubilized with 0.2ml saline just prior to injection.

To prevent initial Alloxan-induced fatal hypoglycemia, the rats were exposed to 10% glucose solution by Oral feeding needle; then they were allowed to 5% glucose in drinking water for the next 24 h. 48 h after Alloxan injection, rats with plasma glucose levels of >140mg/dl were included in the study. Fasting blood glucose levels of rats were checked by glucometer using glucose test

strips. Treatment with plant extract was started 48 h after the alloxan injection.

The treatment was started on the same day except for the normal control and diabetic control groups for 21 days orally. During this period, animals in all groups had free access to standard animal feed and fresh water.

Blood glucose levels were estimated on days 1, 7, 14, and 21 of the study. Also, the body weight of the rats was recorded on days 1, 7, 14, and 21 of the study. On the 21st day, blood samples were collected from overnight fasted rats by retro-orbital plexus under mild ether anesthesia. Blood was collected and allowed to stand for one hour; serum was separated by centrifuging and evaluated for different biochemical parameters.

Biochemical Parameters [38, 39]:
Body weight:
Blood glucose:
Serum glucose levels:
Lipid profile [40, 41, 42, 43, 44]:

- Serum Total Cholesterol Levels.
- Serum Triglycerides Levels.
- Serum HDL Cholesterol Levels.
- Serum LDL Cholesterol Levels.

Kidney Function Tests [45, 46]:

1. Serum Creatinine Levels
2. Serum alkaline phosphatase
3. Serum urea levels

Histopathological studies of the pancreas were also carried out [47, 48]:

RESULTS:

Antioxidant Activity DPPH Free Radical Scavenging: The ability of methanolic and Hydroalcoholic extracts of *Abies pindrow* was calculated by percentage inhibition which was increased with concentration and found to be 86.88% (IC_{50} = 902.39 ±2.15) and 92.09% (IC_{50} = 704.39 ± 2.15) respectively at concentration of 533.33µg/ml, whereas percentage inhibition of Ascorbic acid at the same concentration was 98.16% (LC_{50} 964.39±2.15).

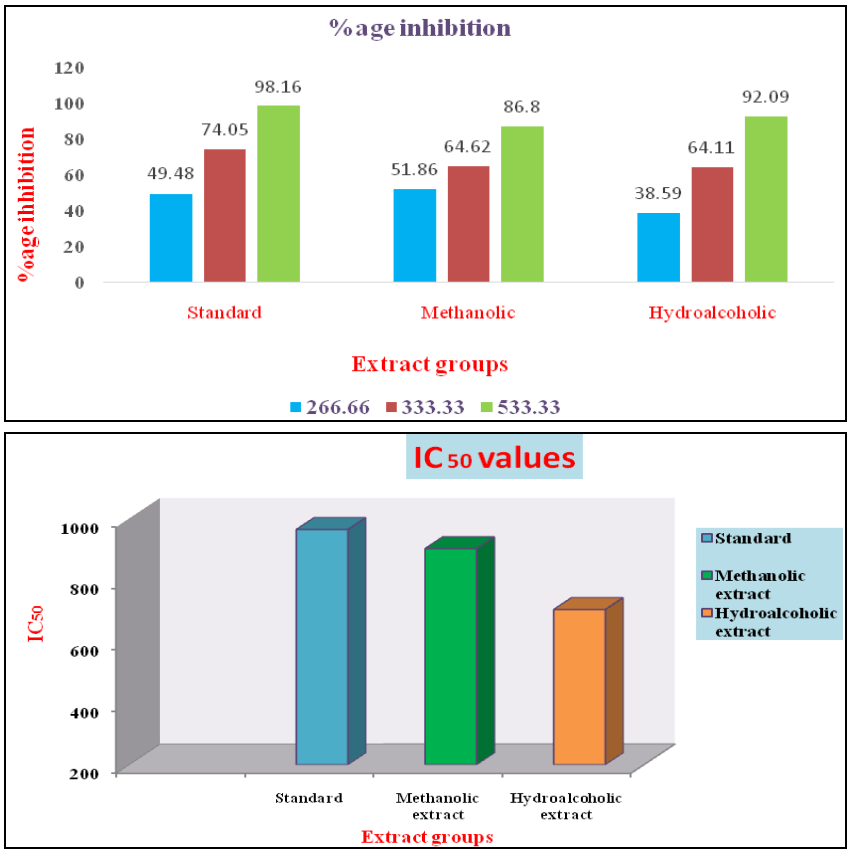

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY AND IC_{50} OF ASCORBIC ACID, METHANOLIC AND HYDROALCOHOLIC EXTRACTS OF ABIES PINDROW LEAVES

Reducing Power: Methanolic and Hydroalcoholic extracts of *Abies pindrow* showed significant reducing power when compared with standard ascorbic acid. The methanolic and Hydroalcoholic showed 0.675 and 0.785 absorbances, respectively, at the concentration of 250µg/ml, when compared to standard Ascorbic acid, which showed absorbance of 0.988 at the same concentration.

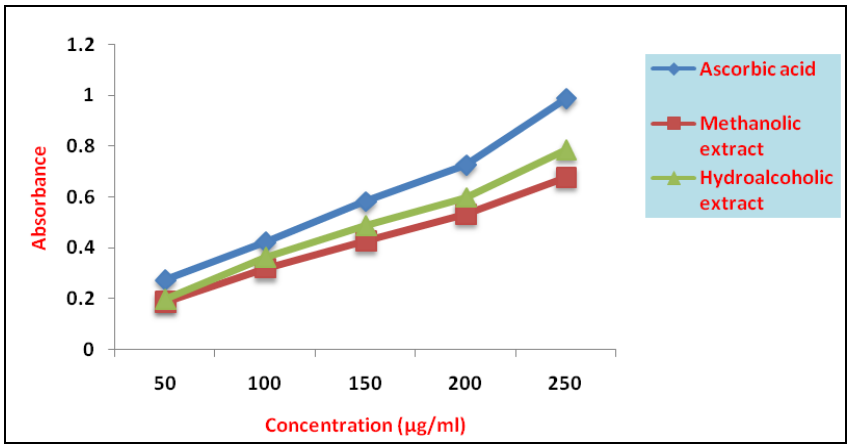

FIG. 2: REDUCING POWER OF ASCORBIC ACID, METHANOLIC, AND HYDROALCOHOLIC EXTRACTS OF *ABIES PINDROW* LEAVES

Total Flavonoid Content: The result of the total Flavonoid content of Methanolic and Hydroalcoholic extract of *Abies pindrow* leaves and the calibration curve of standard Rutin are given below. The total Flavonoid content of Hydroalcoholic and Methanolic extracts in Rutin equivalent was found to be 667mg/g and 517mg/g, respectively.

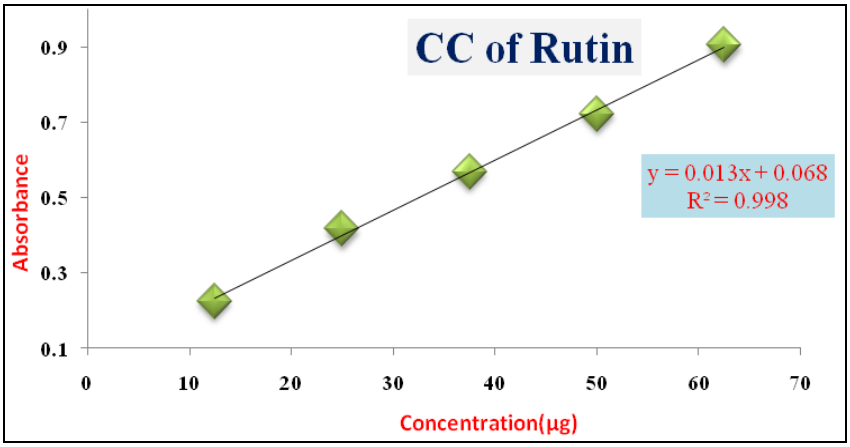

FIG. 3: CALIBRATION CURVE OF RUTIN FOR DETERMINATION OF TOTAL FLAVONOID CONTENT

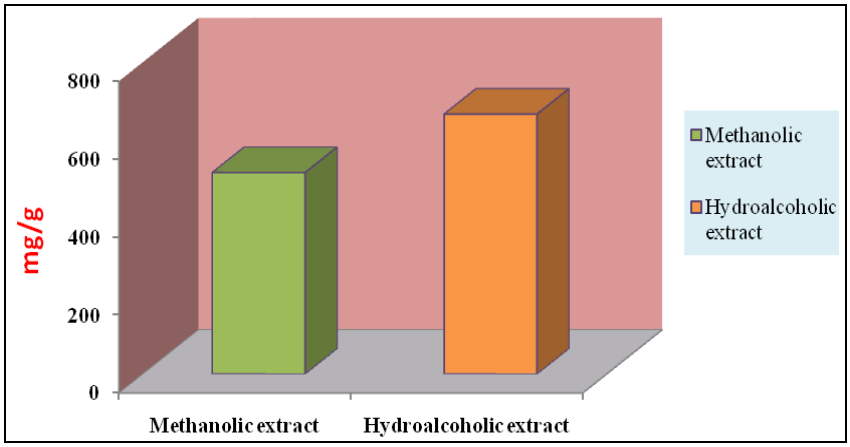

FIG. 4: TOTAL FLAVONOID CONTENT OF METHANOLIC AND HYDROALCOHOLIC EXTRACTS OF *ABIES PINDROW* LEAVES

Total phenolic Content: The result of total Phenolic content of Methanolic and Hydroalcoholic extract of *Abies pindrow* leaves and the calibration curve of standard Gallic acid are given below. The total Phenolic content of Hydroalcoholic and Methanolic extracts in Gallic Acid Equivalents (GAE) was found to be 771mg/g and 719mg/g respectively.

TABLE 1: TOTAL PHENOLIC CONTENT OF METHANOLIC AND HYDROALCOHOLIC EXTRACTS OF ABIES PINDROW LEAVES

Extract	Total Phenolic content(mg GAE/g extract)
Methanolic	719±2.412
Hydroalcoholic	771±3.015

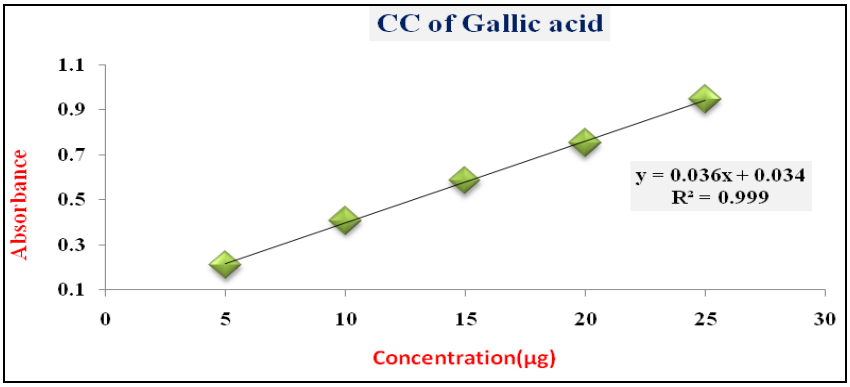

FIG. 5: CALIBRATION CURVE OF GALLIC ACID FOR DETERMINATION OF TOTAL PHENOLIC CONTENT

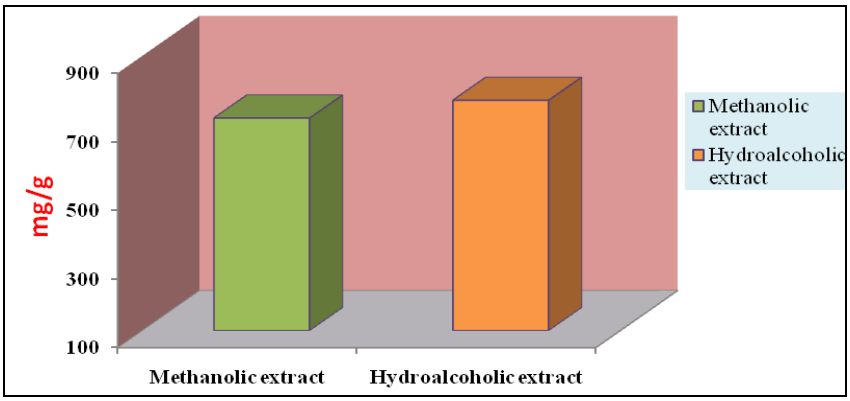

FIG. 6: TOTAL PHENOLIC CONTENT OF METHANOLIC AND HYDROALCOHOLIC EXTRACTS OF *ABIES PINDROW* LEAVES

From the results of Phytochemical and Antioxidant screening,it is clear that among the Methanolic and hydroalcoholic extracts of *Abies pindrow* leaves. The hydroalcoholic extract shows more activity compared to methanolic extract. On this basis, hydroalcoholic extract was selected for the screening of its anti-diabetic potential.

Anti-diabetic Activity of Hydroalcoholic Extract of *Abies pindrow* Leaves: Anti-diabetic studies using alloxan as diabetic model revealed the following results. Effect of different doses of Hydroalcoholic extract of *Abies pindrow* leaves, against alloxan induced Diabetes Mellitus in rats was studied on the following parameters:-

Blood Glucose Levels: (Recorded on day 1,7,14 and 21).

Lipid Profile:

- Serum Total Cholesterol Levels
- Serum Triglycerides Levels
- Serum HDL Cholesterol Levels
- Serum LDL Cholesterol Levels

Kidney Function Tests:

- Serum Urea Levels
- Serum Creatinine Levels

Liver Function test: Serum Alkaline phosphatase

Body Weight: (Recorded on days 1,7,14 and 21)

Statistical Analysis: The data obtained from the different studies and the biochemical estimations are expressed as Mean±SEM for each group.

After this, the statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by a student's test.

Values $p>0.05$ were considered non-significant, $p<0.05$ significant, $p<0.01$ highly significant, and $p<0.001$ very highly significant, respectively.

Biochemical parameters:

Blood Glucose Level (mg/dl):

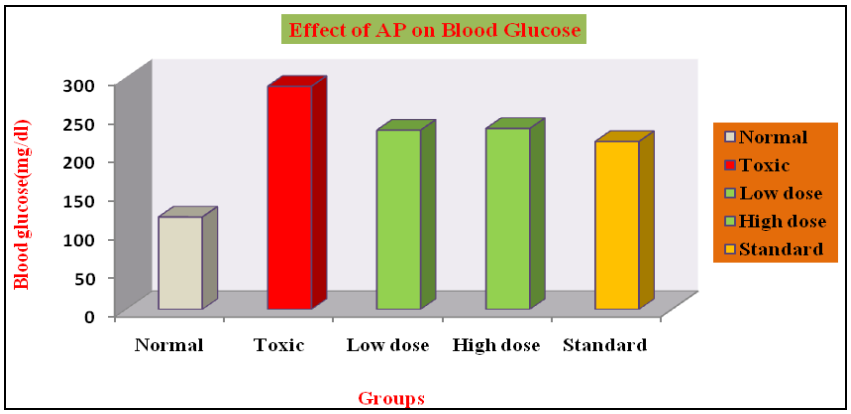

FIG. 7: EFFECT OF DIFFERENT DOSES OF HYDROALCOHOLIC EXTRACT OF AP *ABIES PINDROW* LEAVES (P.O), ON BLOOD GLUCOSE AGAINST ALLOXAN-INDUCED DIABETES MELLITUS IN RATS

Lipid Profile:

Serum Total Cholesterol Levels (mg/dl):

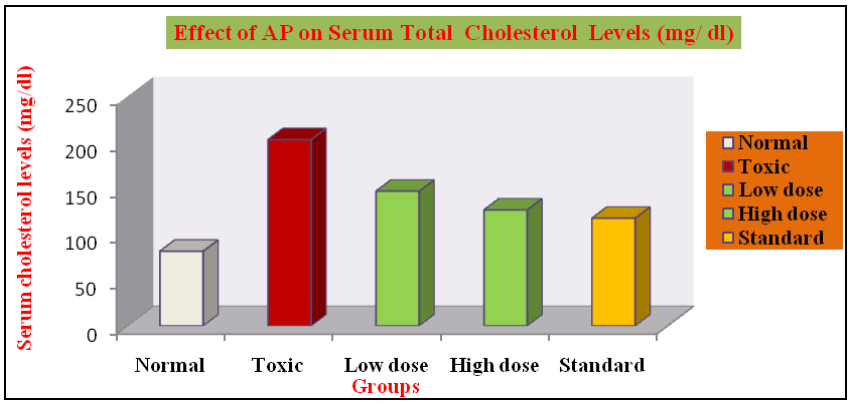

FIG. 8: EFFECT OF DIFFERENT DOSES OF HYDROALCOHOLIC EXTRACT OF *ABIES PINDROW* LEAVES (P.O), ON SERUM CHOLESTEROL LEVELS (MG/DL) AGAINST ALLOXAN-INDUCED DIABETES MELLITUS IN RATS

Serum Triglyceride Levels (mg/ dl):

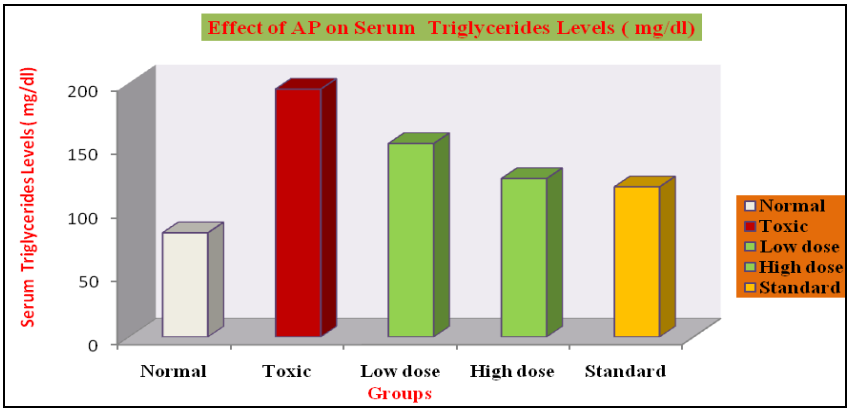

FIG. 9: EFFECT OF DIFFERENT DOSES OF HYDROALCOHOLIC EXTRACT OF *ABIES PINDROW* LEAVES (P.O), ON SERUM TRIGLYCERIDES LEVELS (MG/DL) AGAINST ALLOXAN-INDUCED DIABETES MELLITUS IN RATS

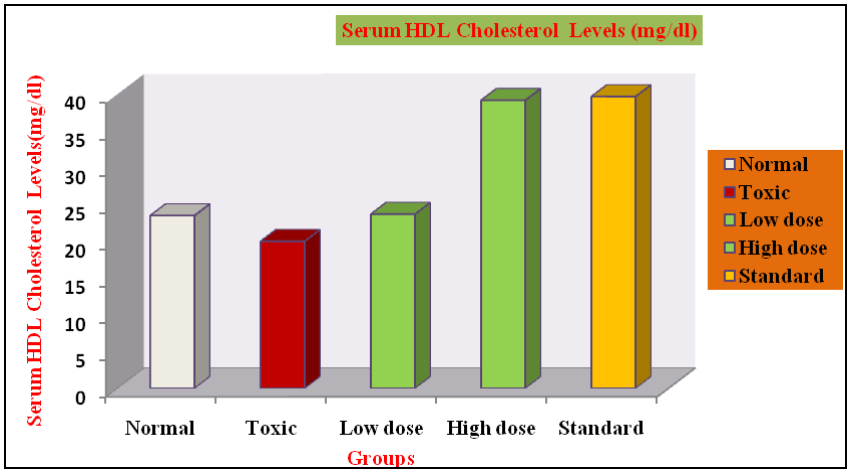

FIG. 10: EFFECT OF DIFFERENT DOSES OF HYDROALCOHOLIC EXTRACT OF *ABIES PINDROW* LEAVES (P.O), ON SERUM HDL CHOLESTEROL LEVELS (MG/DL) AGAINST ALLOXAN-INDUCED DIABETES MELLITUS IN RATS

Serum LDL Cholesterol Levels (mg/dl)

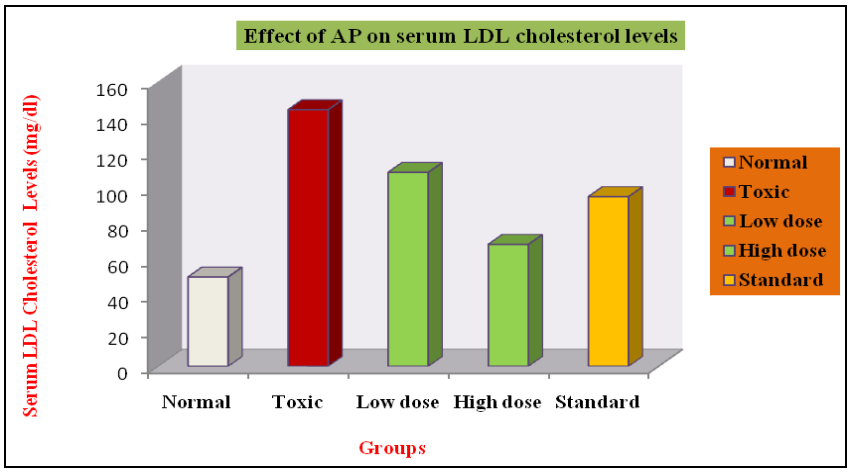

FIG. 11: EFFECT OF DIFFERENT DOSES OF HYDROALCOHOLIC EXTRACT OF *ABIES PINDROW LEAVES* (P.O), ON SERUM LDL CHOLESTEROL LEVELS (MG/DL) AGAINST ALLOXAN-INDUCED DIABETES MELLITUS IN RATS

Kidney Function Tests: Serum Urea Levels (mg/dl)

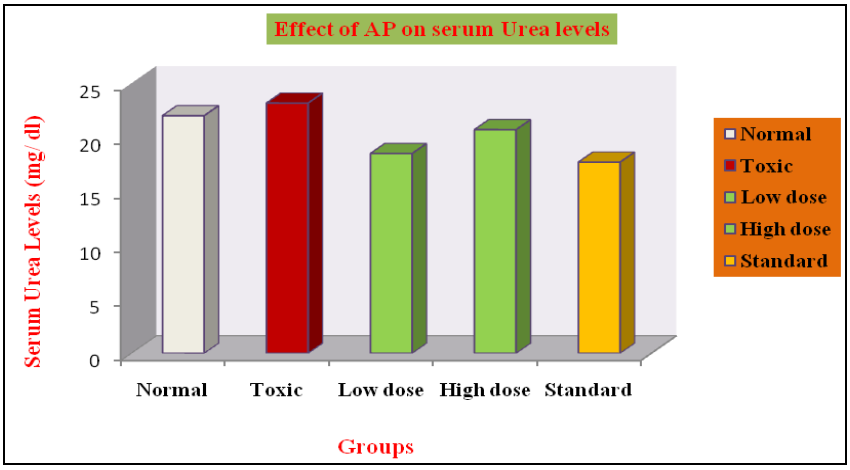

FIG. 12: EFFECT OF DIFFERENT DOSES OF HYDROALCOHOLIC EXTRACT OF *ABIES PINDROW* LEAVES (P.O), ON SERUM UREA LEVELS (MG/DL) AGAINST ALLOXAN-INDUCED DIABETES MELLITUS IN RATS

Serum Creatinine Levels (mg/dl)

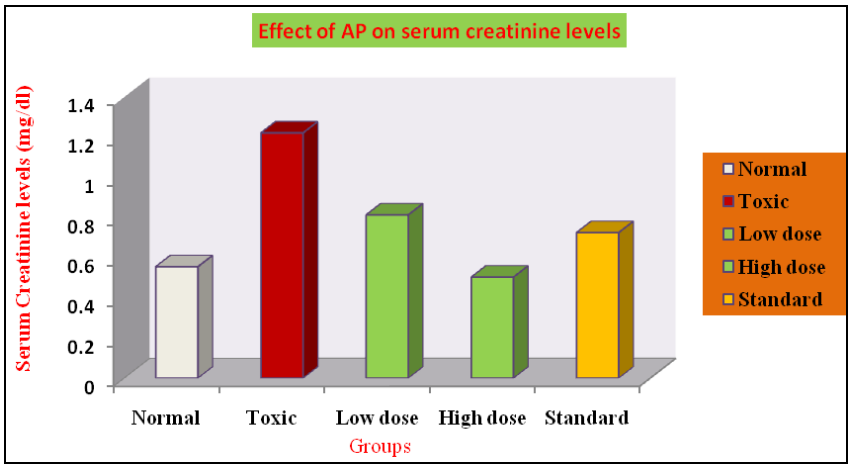

FIG. 13: EFFECT OF DIFFERENT DOSES OF HYDROALCOHOLIC EXTRACT OF *ABIES PINDROW* LEAVES (P.O), ON SERUM CREATININE LEVELS (MG/DL) AGAINST ALLOXAN-INDUCED DIABETES MELLITUS IN RATS

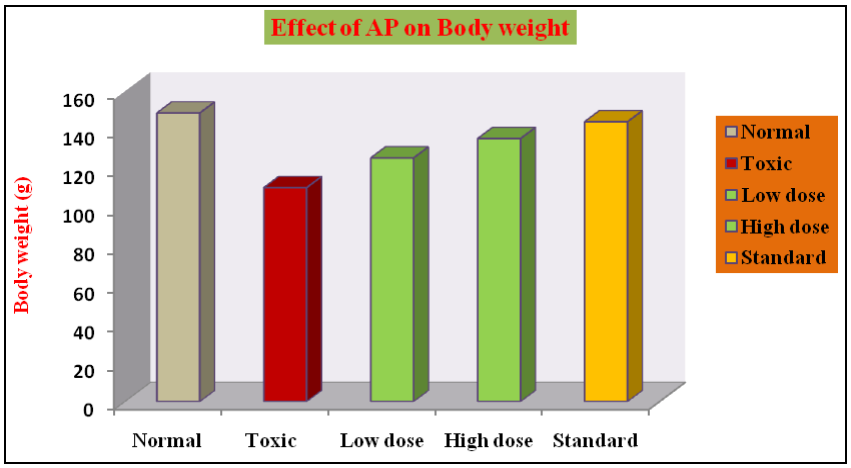

FIG. 14: EFFECT OF DIFFERENT DOSES OF HYDROALCOHOLIC EXTRACT OF *ABIESPINDROW* LEAVES (P.O), ON SERUM ALKALINE PHOSPHATASE (U/L) AGAINST ALLOXAN-INDUCED DIABETES MELLITUS IN RATS

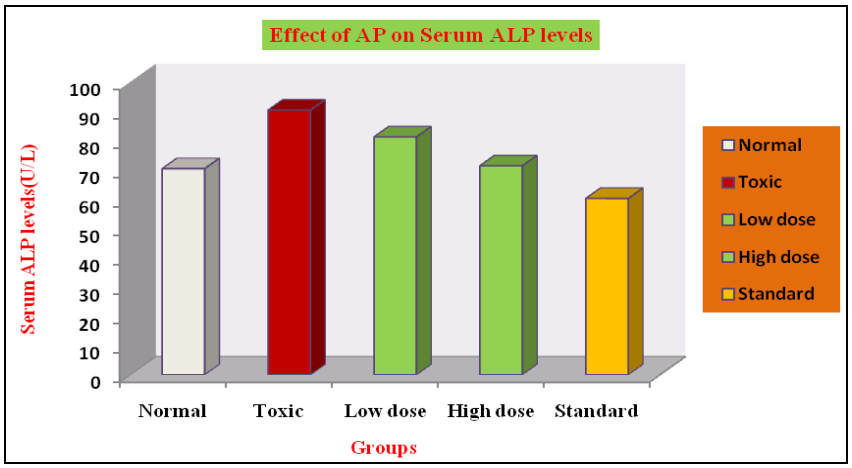

FIG. 15: EFFECT OF DIFFERENT DOSES OF HYDROALCOHOLIC EXTRACT OF *ABIES PINDROW* LEAVES (P.O), ON BODY WEIGHT (G) AGAINST ALLOXAN-INDUCED DIABETES MELLITUS IN RATS

Histopathology of Rat Pancreas:

Effect of Hydroalcoholic Extract of *Abies pindrow* (AP) Leaves on Histopathology of Pancreas in Alloxan Induced Diabetic rats (21 days) are shown by Photomicrographs:

Normal control group (Normal Saline):

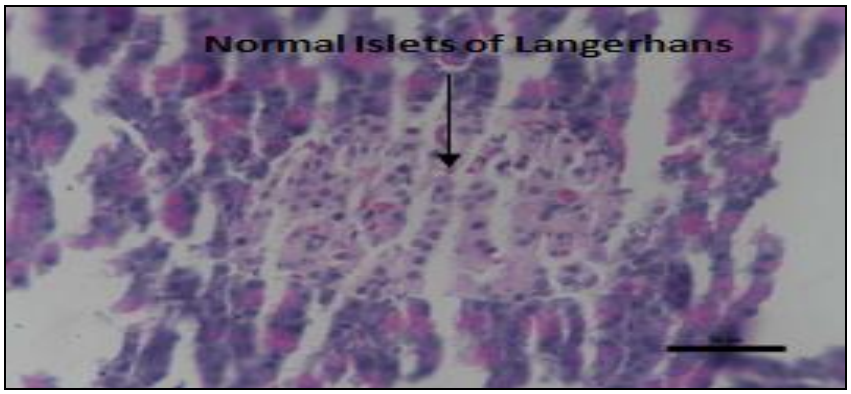

FIG. 16A: PANCREAS OF RATS SHOWING A NORMAL LARGE ISLET

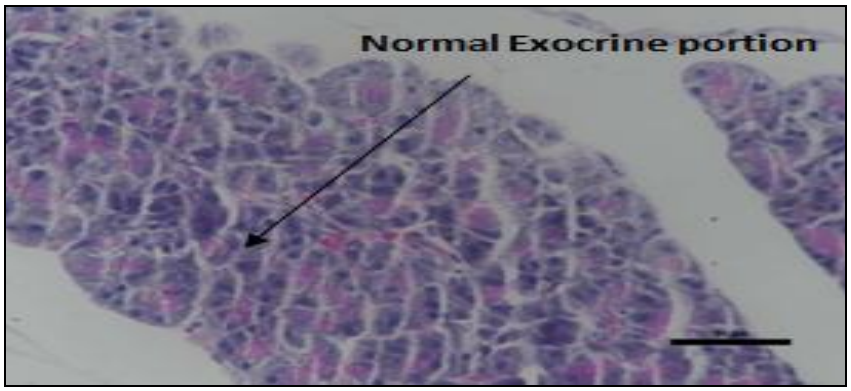

FIG. 16B: PANCREAS OF RATS SHOWING NORMAL EXOCRINE PORTION

Toxic control group (Alloxan 150mg/kg b.w)

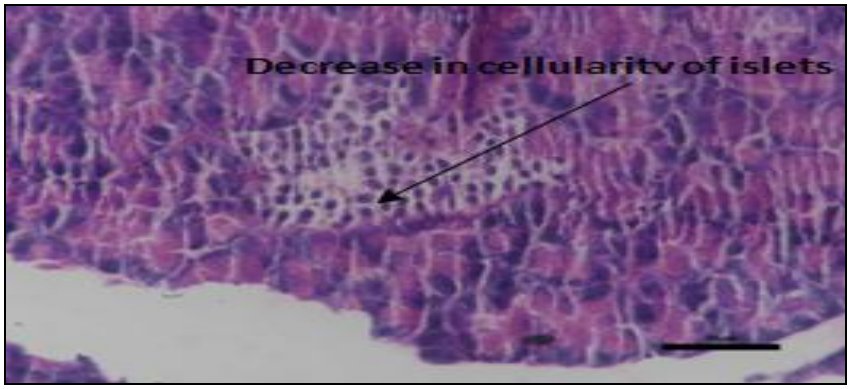

FIG. 17A: PANCREAS FROM DIABETIC RATS SHOWING DEGENERATION OF B-CELLS AND DECREASE IN CELLULARITY OF ISLETS

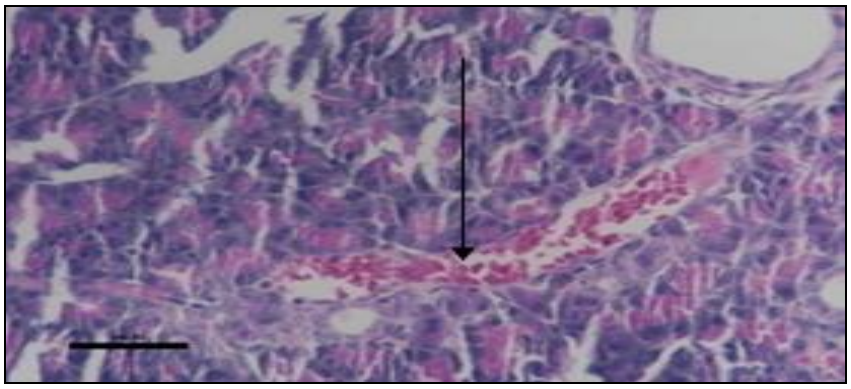

FIG. 17B: PANCREAS FROM DIABETIC RATS SHOWING VASCULAR CONGESTION OF BLOOD VESSELS

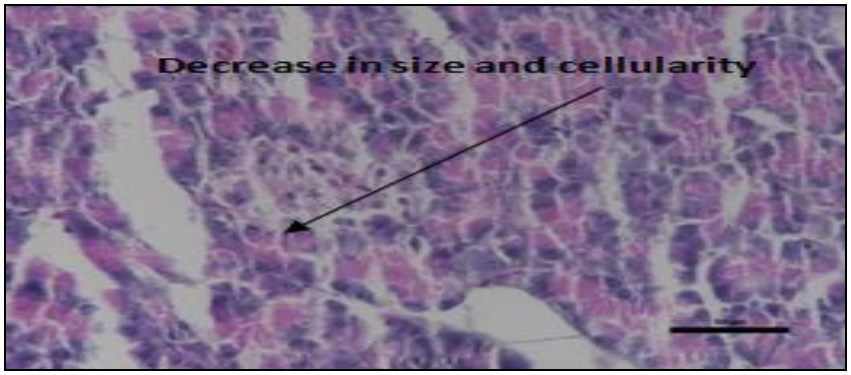

FIG. 17C: PANCREAS FROM DIABETIC RATS SHOWING NECROTIC CHANGES IN B-CELLS, FEW INFLAMMATORY CELLS AND DECREASE IN SIZE OF ISLETS

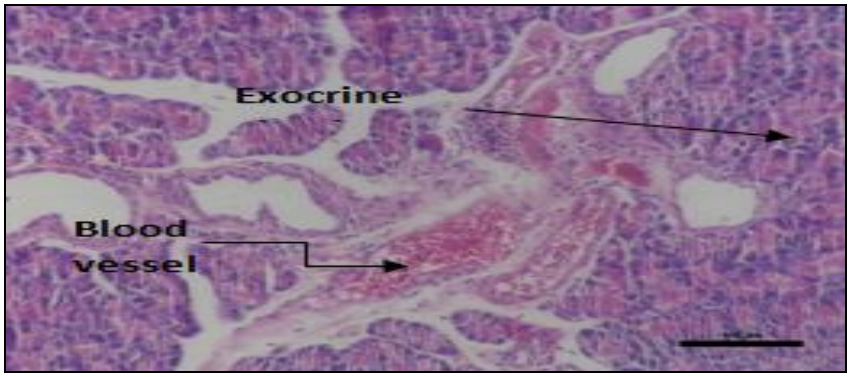

FIG. 17D: PANCREAS FROM DIABETIC RATS SHOWING DILATION OF BLOOD VESSELS AND LYMPHOCYTIC INFILTRATION INTO THE ISLET CELLS

Low Dose group (Alloxan+AP-200mg/kg b.w)

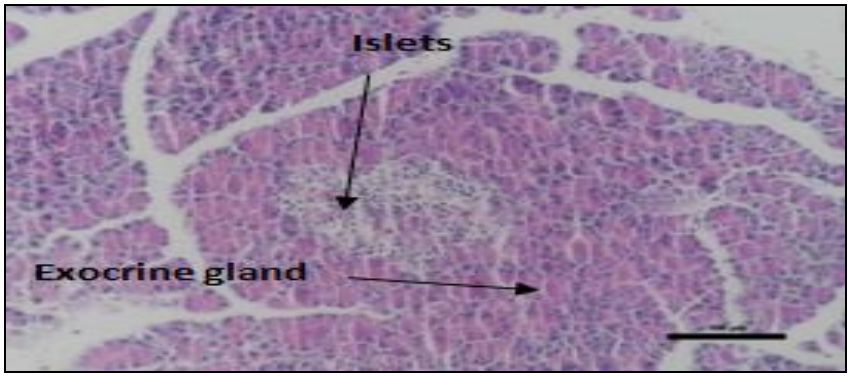

FIG. 18A: PANCREAS FROM DIABETIC RATS SHOWING A COMPARATIVELY NORMAL ISLET STRUCTURE. NO INFLAMMATORY CELLS ARE SEEN IN THE ISLET

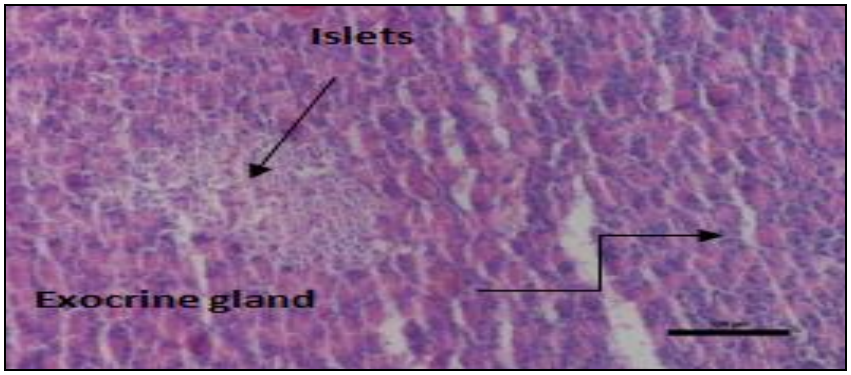

FIG. 18B: PANCREAS FROM DIABETIC RATS SHOWING A COMPARATIVELY SMALLER ISLET STRUCTURE WITH EXOCRINE GLAND TISSUE. VERY FEW INFLAMMATORY CELLS ARE SEEN IN THE ISLET

High Dose group (Alloxan+AP 400mg/kg b.w)

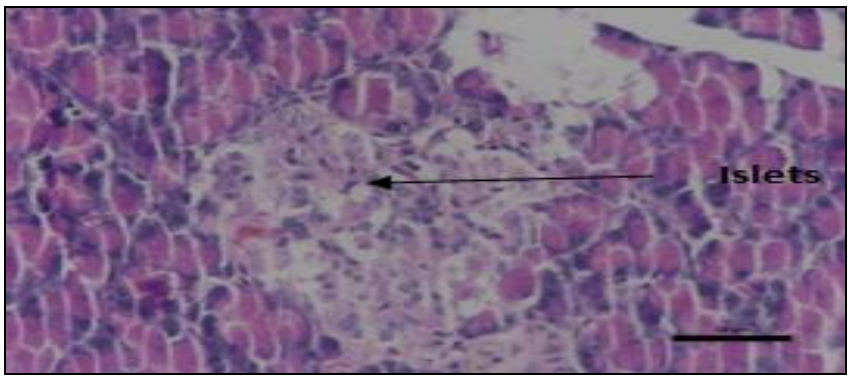

FIG. 19A: PANCREAS FROM DIABETIC RATS SHOWING REGENERATION OF B-CELLS AND INCREASE IN CELLULARITY OF ISLETS

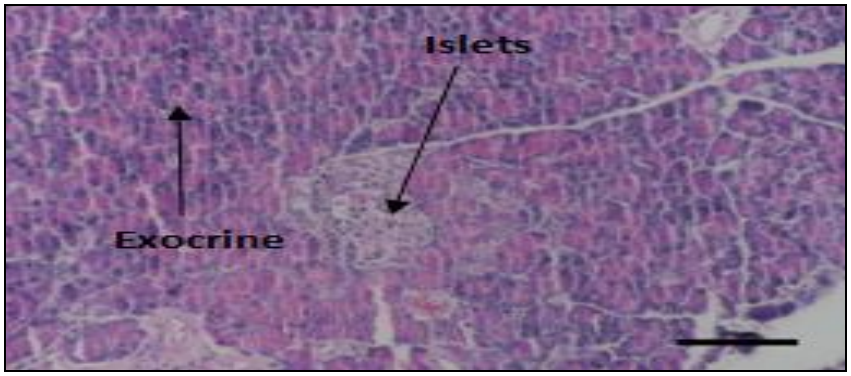

FIG. 19B: PANCREAS FROM DIABETIC RATS SHOWING COMPARATIVELY SMALLER ISLET STRUCTURE WITH EXOCRINE GLAND TISSUE AND NOINFLAMMATORY CELLS ARE SEEN IN THE ISLETS

Standard group (Alloxan+Glibenclamide-5mg/kgb.w)

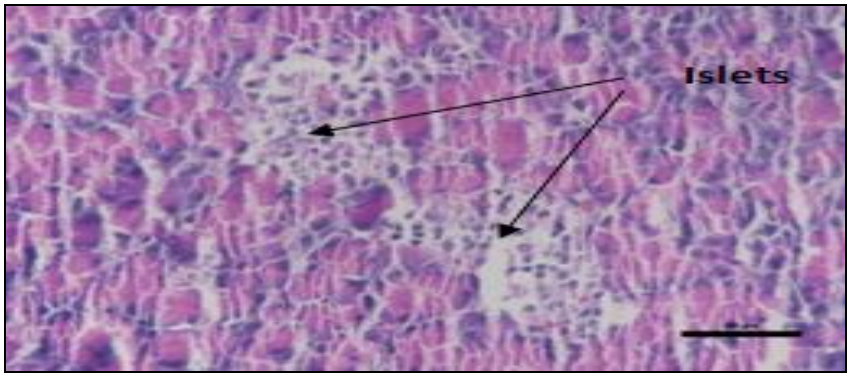

FIG. 20A: PANCREAS FROM DIABETIC RATS SHOWING REGENERATION OF B-CELLS AND NORMAL ISLET STRUCTURE

FIG. 20B: PANCREAS FROM DIABETIC RATS SHOWING INCREASE IN SIZE AND CELLULARITY OF ISLETS OF LANGERHANS

Histopathological examination of all the groups show the following changes:

Histopathological studies of the pancreas of the slides of rats of the Normal control group show a large islet structure surrounded by exocrine gland tissue. No inflammatory cells are seen in the islet. Proper cellularity and size are seen in islets of langerhans and exocrine glands **Fig. 16AB**. The pancreas from the slides of the Toxic control group shows a decrease in the cellularity of islet structure and degeneration of β-cells **Fig 17A**. There is also vascular congestion of blood vessels **Fig. 17B** necrotic changes in β- cells, few inflammatory cells, and a decrease in the size of islets **Fig. 17C**. There is dilation of blood vessels and lymphocytic infiltration into the islet cells **Fig. 17D**.

Low dose (200mg/kg) of *Abies pindrow* (AP) extract administered to low dose Group showing a comparatively normal islet structure surrounded by exocrine gland tissue. No or very few inflammatory cells are seen in the islet **Fig. 18 AB**.

High dose (400mg/kg) of AP extract administered to high dose Group showing partial restoration of normal cellular population β-cells and increase in cellularity of islets of Langerhans **Fig. 19A**. There is smaller islet structure surrounded with exocrine gland tissue and no inflammatory cells are seen in the islets **Fig. 19B**. Glibenclamide (Standard anti-diabetic drug) when administered at the dose level of 5 mg/kg b.w to rats of Standard control Group showing restoration of normal cellular population, enlarged size of β-cells **Fig. 20 AB**. There is an increase in the size and cellularity of islets of Langerhans.

DISCUSSION AND CONCLUSION: Present study elucidated the phytochemical, Antioxidant and Anti-diabetic potential of *Abies pindrow* (AP) leaves extract.

- Our results deciphered that among the Methanolic and Hydroalcoholic extracts of AP leaves, Hydroalcoholic extract exhibit significant amount of bioactive compounds *viz* phenols, flavonoids, alkaloids, tannins, steroids, *etc* and having excellent Antioxidant potential.

- Phenolics have been proven to determine their potential in preventing β-cell apoptosis, promoting β-cell proliferation and insulin secretion, and enhancing insulin sensitivity activity; on this basis, Hydroalcoholic extract of AP leaves was evaluated for antihyperglycemic activity.

- Among different doses of Hydroalcoholic extract of the leaves of AP, the dose of 400 mg/kg b.w has produced a highly significant fall in fasting blood glucose levels and an increase in the body weight of alloxan-induced diabetic rats, indicating that AP leaves have significant antihyperglycemic activity.

- Biochemical parameters such as Urea and Creatinine were significantly increased in Diabetic induced rats. In contrast, when treated with Hydroalcoholic extract of AP leaves, the increased levels were significantly decreased to that of the normal indicating significant Anti-diabetic activity.

- Lipid profiles such as cholesterol, triglycerides, and LDL were significantly increased, whereas HDL levels decreased significantly in Diabetic induced rats. When treated with Hydroalcoholic extract of AP leaves, the levels were reversed to that of the normal levels.

- The serum marker enzymes such as ALP were significantly increased in Diabetic induced rats, whereas when treated with Hydroalcoholic extract of AP leaves, the levels were restored to that of normal, indicating its significant Anti-diabetic activity.

- Histopathological examination revealed that the pancreas of Diabetic untreated rats showed features of insulitis with lymphocytic infiltrations, vascular congestion and destruction of β- cells, where as the pancreas of Diabetic rats treated with Hydroalcoholic extract of *Abies pindrow* leaves showed the normal architecture of tissue cells indicating its regenerative effect. Treatment with glibenclamide also produced similar changes.

All these results confirm that the leaves of *Abies pindrow* possess Anti-diabetic activity and their Phytochemicals, such as flavonoids and other phenolics compounds, could be the active principles responsible for the Anti-diabetic and

other beneficial effects. Extensive research should be performed to isolate the main constituents responsible for this activity and elucidate the other mechanisms of action of the Anti-diabetic activity.

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