RESEARCH ARTICLE

Ameliorative Potential of *Abies Pindrow Royle* Extract on Dysregulated Biochemical Parameters in Type-II Diabetic Wistar Rats: An Experimental Investigation

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Received: 7th Oct, 2025; Revised: 12th Jan; Accepted: 10th Feb; Available Online: 15th Mar, 2026

ABSTRACT

Objective: The ethanolic extract of *Abies Pindrow Royle* leaves extract shown antidiabetic activity at different concentration in rodents was determined in comparison to Glibenclamide (standard group), vehicle group, normal control.

Materials and Methods: The leaves of *Abies Pindrow Royle* were collected from Gulaba-Kothi, Himachal Pradesh, and authenticated by a plant taxonomist. The extracts were prepared using ethanol and administered to Wistar rats in doses of 150, 300, and 600 mg/kg. All analytical grade chemicals used in this study were purchased from certified suppliers. Experimental protocols were approved by the IAEC of MDU, Rohtak. Diabetes was induced in rats using standard procedures, and the treatment groups received either the plant extract or Glibenclamide (10 mg/kg).

Results: The ethanolic extract of leaves of *Abies Pindrow Royle* plant showed antidiabetic activity by its action on Glucokinase enzyme and enhanced the glucose metabolism by increasing the conversion of glucose into glucose-6-phosphate. The results suggested that *Abies Pindrow Royle* plant extract at dose of 300 mg/kg has potential to diminish the level of glucose.

Conclusion: The findings suggest that *Abies Pindrow Royle* leaf extract, particularly at a dose of 300 mg/kg, possesses strong anti-diabetic properties and moderate anti-nociceptive effects. Its mechanism may involve stimulation of Glucokinase, contributing to improved glucose regulation. Thus, this plant extract holds promise as a potential natural alternative for managing diabetes and related complications. Further studies are warranted to elucidate its exact mechanism of action and long-term safety.

Keywords: Antidiabetic, Ethanolic Extract, Glucokinase, Abies Pindrow, Streptozotocin etc.

How to cite this article: Chhikara M, Bhatt A, Duhan M, Mittal C, Sharma B, Chourasia V, Singh M, Anand S, Rana S, Narwal S. Ameliorative potential of abies pindrow royle extract on dysregulated biochemical parameters in type-ii diabetic wistar rats: an experimental investigation. Int J Drug Deliv Technol. 2026;16(9s): 542-548; Doi: 10.25258/Ijddt.16.9s.57

Source of support: Nil

Conflict of interest: None

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INTRODUCTION

Diabetes mellitus (DM) reported as group of diseases that occurred during that period when the pancreas does not produce adequate amount of insulin[1]. Degradation in level of insulin or resistance of insulin within the body by effect in gall tissues like muscles of skeletal, liver, adipose tissue, genes, effectors enzymes, signal transduction system and insulin receptors are liable for the deficiency in metabolism[2]. Deficiency of insulin and resistance of insulin are occurring by the autoimmune damage of the pancreatic β-cells, action on target tissue by the abnormalities occurs in fat, protein, and carbohydrate metabolism[3]. In diabetes long term complication includes retinopathy (loss of vision, glucoma), osteoporosis (bone disease), nephropathy (renal failure), neuropathy (foot ulcers, neuropathic arthropathy[19], amputations) and autonomic neuropathy (dysfunctions in the nerves that regulate non-voluntary body functions like sweating, blood pressure, heart rate etc.), gastrointestinal(GI), genitourinary, and cardiovascular (CVS) symptoms, sexual dysfunction[4]. In DM increase risk of CVD, High blood pressure (heart attack), stroke (clot blood vessel) and abnormalities of lipoprotein metabolism[5]. Diabetes neuropathy is a degeneration of neurons that's result perception of pain lost in DM rats. Pain perception in rats will be measured by tail flick method and eddy's hot plate methods. Through nerve impulse brain send message to the pancreas and other organs. Hyperglycemia decreases the effect of nerve conduction into the brain[7-9]. Treated DM rats were improve their body weight due to tissue protein increment. Due to unavailability of carbohydrate degrade or catabolized the structural proteins. Increase the energy metabolism decrease the body weight in rodents.

In this article, the author evaluated diabetic activity of ethanolic extract of A*bies pindrow Royle* plant leaves extract and also determined the mechanism by which *Abies pindrow Royle* plant leaves have anti-diabetic activity. The results suggest that *Abies pindrow Royle* plant extract (300 mg, /kg) dose was found more effective in the treatment of diabetes by diminished the level of glucose.

METERIALS AND METHODS

Drugs: The analytical grade chemicals are used in this study and all chemicals were purchased from Sigma Aldrich, Pvt. Ltd., Mumbai, Himedia Chemicals Pvt. Ltd., Mumbai.

Collection and Preparation of plant extract

The leaves of *Abies pindrow Royle* were collected from Gulaba Kothi, HP, India. and authenticated and identified by Dr. Surender, Department of Botany, M.D.U., Rohtak, India. The leaves of plant firstly washed with dist. H_2O. Leaves were shade dried for two weeks, powdered and were passed, through sieve No. 60 for extraction. Powdered materials of plant were extracted through soxhlet apparatus in 90% ethanol at temperature of 50^0C to 60^0C. The solvent was removed through the residue by reducing the pressure. The yield of plant extract will be found out 8.21 %. The residue has been dried in desiccator and stored in refrigerator at 4^0C for further use.

Rats were obtaining by CAH (Central Animal House) MDU, Rohtak from LUVAS' animal house, Hisar, Haryana, India after approval in meeting held by MDU with IAEC (Institutional Animal Ethics Committee in MDU, Haryana India via letter no. 1767/RE/S/14/CPCSEA on dated 31/08/2017.

Fig.1 Plant leaves and extraction method.

Toxicity evaluation: The acute oral toxicity assessment was performed following the OECD Guideline No. 423. A single oral dose of 600 mg/kg body weight of the ethanolic extract of *Abies pindrow Royle* was administered, to three rats. The animals, were closely monitored over a 14-day period for any signs of toxicity, changes in behavior, or mortality. To validate the results and confirm the classification of acute toxicity, the test was replicated in another group of three rats. This approach facilitated the estimation of the median lethal dose (LD_{50}) and confirmed the safety profile of the extract at the given dose.

Animals Used:

150-180 gm. Male wistar rats are corroborated under standard conditions such as natural day, and night cycle, temperature 25. ± 2°C and humidity 60-70%

and had free reach to standard feed and fresh water for two weeks. After two weeks, rats were divided randomly into 6 groups, (n=6). The mice were kept in polycarbonate cages (6 animals per cage) under required conditions with free access of feed and water. As per CPCSEA guidelines all animals were handled.

Doses selection: - The dosages of various drugs were determined based on evidence and references available in previously published literature i.e. Glibenclamide (10mg/kg), Streptozotocin (60mg/kg), Nicotinamide (110mg/kg) and *Abies pindrow Royle* plant extract (150mg/kg, 300mg/kg, 600mg/kg).

Experimental groups: -

Groups (n=6) that will be used for the assessment of following activities

Sr. no.	Groups	Administration of dose
1.	Group – 1	Administration of 0.9% sodium chloride solution to normal control rats
2.	Group – 2	Vehicle group received 0.9% NaCl
3.	Group – 3	Administration of glibenclamide 10mg/kg to the standard group
4.	Group – 4	*Abies pindrow Royle* extract 150mg/kg administered to this group
5.	Group– 5	Administration of *Abies pindrow Royle* extract 300mg/kg to this group
6.	Group – 6	*Abies pindrow Royle* leaf extract 600mg/kg administration to this group

Abies pindrow Royle extract and glibenclamide standard drug will be suspended on 0.9% sodium chloride in normal water by oral routes for 28 days.

Induction of Diabetes Mellitus: -

Before causing diabetes mellitus, rats were fasting overnight. Diabetes mellitus is induced through intra peritoneal injection (STZ). Streptozotocin dissolved at pH 4.5 in 0.1 M cold sodium citrate buffer[26]. The dose of the STZ will be 60mg/kg diabetic control rats received alone vehicle. To overcome the drug induced hyper glycaemia used to drink 5% sucrose solution to the animals. After 4th day of diabetes induction development of diabetes will be confirmed. The hyperglycemia and glycosuria (moderate diabetes) in the rats having the blood glucose level will be elevated at 190mg/dl and these rats are considered the diabetic rats. These rats are used in experimental study[10-13].

Analgesic models: - The analgesic activity was measured by using the eddy's hot plate and tail immersion methods after 4 weeks of STZ induced diabetes mellitus.

Statistical analysis

The results were shown in mean+ SEM. By using the one and two-way ANOVA all statistical evaluation was done and compared with Tukey's multiple test for post hoc analysis. GraphPad InStat software was used for data interpretation. Significance levels were determined based on p-values, with thresholds set at $p<0.05$, $p<0.01$, and $p<0.001$.

Result-

A) Analgesic models: - Control: Normal saline; Vehicle: Diabetic control; Std.: Standard drug; PE 150: plant extract 150. mg/kg; PE 300: plant, extract 300. mg/kg; PE 600: plant extract 600 mg/kg.

1. Weight of animals

Weight of diabetic animals (vehicle groups) decreased significantly from day 7 (137.83g) to day 28 (102.5g). A significantly increased in weight of control group was found from day 7(186.17g) to day 28 (237.33g). Observed weight of standard group and different doses of plant extract administered groups show increase significantly ($p<0.001$) as compared to vehicle. Plant extract 300mg/kg treated group found better restoration against decrease in weight as compared to plant extract 150mg/kg and 600mg/kg as represented in fig. 1.

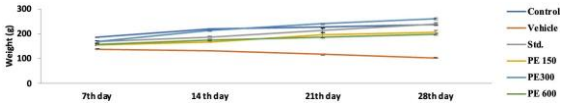

Fig.1. Representation of weight of rats at different time interval

2. Foot withdrawal of animals

Foot withdrawal was measured in hot plate test. The sensitivity deterioration causes significantly increased in foot withdrawal response time of diabetic animals (vehicle group) from day 7 (9 sec.) to day 28 (11.5 sec.). Foot withdrawal time of control group decreased significantly from day 7(1.667sec.) to day 28(2.5 sec.). Due to sensitivity enhanced, foot withdrawal latency time of standard drug treated group and different doses of plant extract treated groups (150, 300 and 600mg/kg) decreased as

compared to vehicle group. Plant extract 300mg/kg dose (3.0 sec.) not show significant difference in foot withdrawal latency on day 28 as compared to standard group (3.5 sec.). as represented in fig. 2.

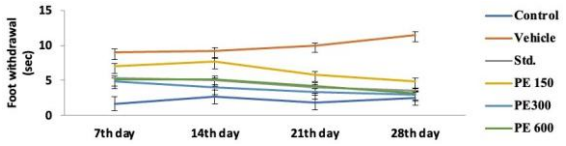

Fig. 2. Representation of foot withdrawal of rats at different time interval

3. Tail flick latency of animals

Tail flick latency was measured by tail immersion test. Due to sensitivity deterioration tail in flick latency time of diabetic animals (vehicle group) increased significantly from day 7 (9.5 sec.) to day 28 (11 sec.). Tail flick latency time of control group decreased significantly from day 7(1.667sec.) to day 28(2.5 sec.) Due to sensitivity enhanced cause decrease in tail flick latency time of standard group at 10mg/kg and plant extract at different doses (150, 300, 600mg/kg) treated groups as compared. to vehicle group, Plant extract 300mg/kg was found better due to significantly decrease. ($p<0.001$) in tail flick latency latency on day 28(2.5 sec.) as compared to standard group (4.167 sec.) as represented in fig. 3.

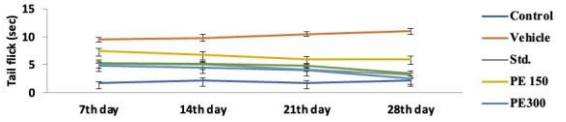

Fig. 3. Representation of tail flick of rats at different time interval

4. Blood glucose level of animals

Glucose level in blood was significantly elevation in diabetic animals (vehicle group) from day 7 (360.8mg/dl) on day 28 (426mg/dl). Glucose level in blood was found to be same in control group from day 7 (108mg/dl) on day 28 (108.33mg/dl). On day 28, glucose level in blood were significantly decreased ($p<0.001$) at different doses of plant extract (150, 300, 600mg/kg) and standard drug 10mg/kg as compared to vehicle group. Plant extract 300mg/kg (126.83mg/dl) show significant decreased, ($p<0.01$) in blood glucose level as compared to standard group (148mg/dl), plant extract 150mg/kg (165.17mg/dl) and 600mg/kg(189.17mg/dl) on day 28 as represented in fig. 4.

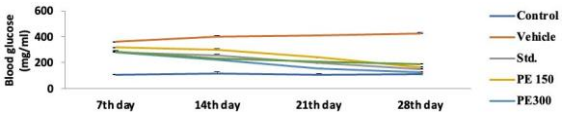

Fig. 4. Representation of blood glucose level of rats at different time interval

B) Experimental study: -Procedure- Values are expressed, as mean ± S.E.M, n=6 in each group. The results were shown in mean+ SEM. By using the one and two-way ANOVA all statistical evaluation was done and compared with Tukey's multiple test for post hoc analysis. test; a: $p<0.001$ v/s control; b: $p<0.001$ v/s vehicle; c: $p<0.001$ v/s standard; d: $p<0.001$ v/s plant extract 150mg/kg; Control (Normal saline); Vehicle: (diabetic control); Std.: standard drug; PE 150: plant extracts 150 mg. /kg; PE 300: plant extracts, 300 mg/kg; PE 600: plant extract 600 mg/kg.

5. Triglyceride level

Triglyceride level in blood of diabetic control (vehicle group) increased significantly at ($P<0.001$) from day 28 (205.13mg/dl). Triglycerides level of control group was decreased significantly at ($p<0.001$) on day 28 (91mg/dl) as compared to diabetic animals (vehicle group) and all treated groups. Triglycerides level in blood of standard drug 10mg/kg treated group decrease significantly at ($P<0.001$) from day 28 (114.3mg/dl) as compared to diabetic animals (vehicle group) and plant extract 300mg/kg. Triglycerides level in blood of plant extract 150mg/kg treated group decrease significantly at ($P<0.001$) from day 28 (137.98mg/dl) as compared to diabetic animals (vehicle group), plant extract 300mg/kg and 600mg/kg. Triglycerides level in blood of plant extract 300mg/kg treated group decrease significantly at ($P<0.001$) from day 28 (102.83mg/dl) as compared to diabetic animals (vehicle group). Triglycerides level in blood of plant extract 600mg/kg treated group decrease significantly at ($P<0.001$) from day 28 (123.93mg/dl) in comparison of diabetic animals (vehicle group) as represented in fig. 5.

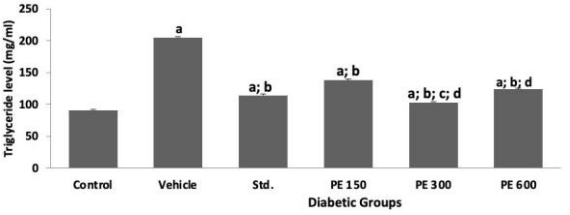

Fig. 5. Effect of various treatments on triglyceride level (mg/dl) in rats on day 28th

6. Total cholesterol

Total cholesterol level in blood of diabetic control (vehicle group) increased significantly at (P<0.001) from day 28 (227.27mg/dl). Total cholesterol level of control group was decreased significantly at (p<0.001) on day 28 (118.9mg/dl) in comparison of diabetic animals (vehicle group) and all treated groups. Total cholesterol level in blood of standard drug 10mg/kg treated group decrease significantly at (P<0.001) from day 28 (135.28mg/dl) as compared to diabetic animals (vehicle group), plant extract 150mg/kg and plant extract 600mg/kg. Total cholesterol level in blood of plant extract 150mg/kg treated group decrease significantly at (P<0.001) from day 28 (161.53mg/dl) as compared to diabetic animals (vehicle group). Total cholesterol level in blood of plant extract 300mg/kg treated group decrease significantly at (P<0.001) from day 28 (127.45mg/dl) as compared to diabetic animals (vehicle group), standard drug 10mg/kg and plant extract 600mg/kg. Total cholesterol level in blood of plant extract 600mg/kg treated group decrease significantly at (P<0.001) from day 28 (143.05mg/dl) as compared to diabetic animals (vehicle group) as represented in fig. 6.

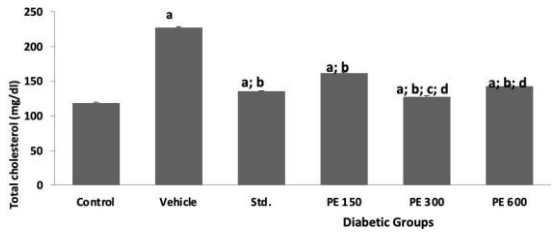

Fig. 6. Effect of various treatments on total cholesterol in rats on day 28th

7. Creatinine level

Creatinine level in blood of diabetic control (vehicle group) increased significantly at (P<0.001) on day 28 (4.979mg/dl). Creatinine level of control group was decreased significantly at (p<0.001) on day 28 (1.536mg/dl) as compared to diabetic animals (vehicle group) and all treated groups. Creatinine level in blood of standard drug 10mg/kg treated group decrease significantly at (P<0.001) from day 28 (1.918mg/dl) as compared to diabetic animals (vehicle group), plant extract 150mg/kg. Creatinine level in blood of plant extract 150mg/kg treated group decrease significantly at (P<0.001) from day 28 (2.069mg/dl) as compared to diabetic animals (vehicle group) and plant extract 300mg/kg. Creatinine level in blood of plant extract 300mg/kg treated group decrease significantly at (P<0.001) from day 28 (1.844mg/dl) as compared to diabetic animals (vehicle group). Creatinine level in blood of plant extract 600mg/kg treated group decrease significantly at (P<0.001) from day 28 (1.974mg/dl) in comparison to diabetic animals (vehicle group) as represented in fig. 7.

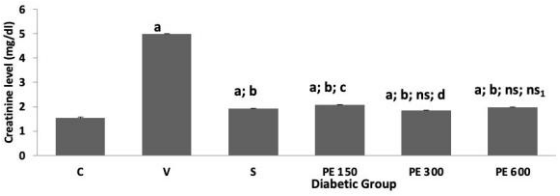

Fig. 7. Effect of various treatments on creatinine level in rats on day 28th

8. VLDL level on day 28th

VLDL level in blood of diabetic control (vehicle group) increased significantly at (P<0.001) from day 28 (47.333mg/dl). VLDL level of control group was decreased significantly at (p<0.001) on day 28 (17.75mg/dl) as compared to diabetic animals (vehicle group) and all treated groups. VLDL level in blood of standard drug 10mg/kg treated group decrease significantly at (P<0.001) from day 28 (31.267mg/dl) as compared to diabetic animals (vehicle group), plant extract 150mg/kg and plant extract 600mg/kg. VLDL level in blood of plant extract 150mg/kg treated group decrease significantly at (P<0.001) from day 28 (35.017mg/dl) as collated to diabetic animals (vehicle group). VLDL level in blood of plant extract 300mg/kg treated group decrease significantly at (P<0.001) from day 28 (23mg/dl) as compared to diabetic animals (vehicle group), standard drug 10mg/kg, plant extract 150mg/kg and plant extract 600mg/kg. VLDL level in blood of plant extract 600mg/kg treated group decrease significantly at (P<0.001) from day 28 (33.633mg/dl) in comparison to diabetic animals (vehicle group) as represented in fig. 8.

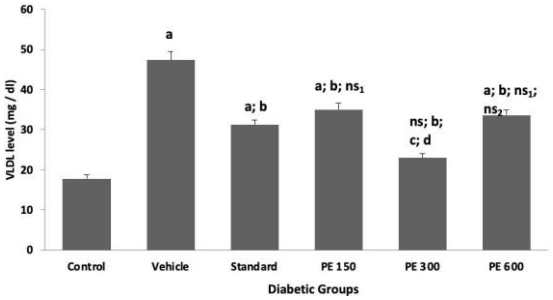

Fig. 8. Effect of various treatments on VLDL level in rats on day 28th

DISCUSSION

Various Indian plants are effective in diabetes mellitus treatment for example plant *Abroma augustum*

(family- Sterculiaceae) acts by decrease blood glucose, *Acacia Arabica* (family- Leguminosae) acts by intiation of insulin release, *Allium sativum* (family- Liliaceae) acts by decrease in blood glucose level[11], *Camellia sinensis* (family- Theaceae) acts by increase in insulin level, *Caesalpinia bonducella* (family- Leguminosae) act by free radical scavenging and *Catharanthus roseus* (family- Apocynaceae) acts by increase in metabolism of glucose etc[14].Different exracts of *Abies pindrow* leaves as petroleum ether, benzene, chloroform, acetone and ethanolic extract 50- 200 mg/kg, i.p. or 200mg/.kg p.o. administration showed significant anti - inflammatory activity after 40 min of administration and in both acute and subacute models 200 mg/kg dose showed anagelsic or ulcerogenic activity in rats. Abies *pindrow Royle* ethanolic. extract doses 100., 200 and 400. mg/kg p.o. in mice show antianxiety activity as comparison to control and 200, 400 mg/kg shows statistically equal anxiolytic effect to the standard drug[13], (2mg/kg i.p.). In present study rats orally treated, with the extracts of Abis pindrow (ethanolic extracts doses 150, 300 and 600. mg/kg, p.o.) were found to be anti - diabetic by significantly depletion in blood glucose level as collated to vehicle group. The plant extract (300 mg/kg, p.o.) dose have meaningful antidiabetic response as compared to standard drug treated group (glibenclamide 10 mg/kg, p.o.).

By STZ drugs induction of DM shows hyperlipidemia and creatinine level in rats[10]. However, the extract treated rats (ethanolic extract dose 300 mg/kg) showed reduction, in LDL, TC, Creatinine and cholesterol in lipid profiles, and creatinine level in serum along with reduction in blood glucose level[11-12,16].

STZ intercepts synthesis of DNA in mammals and bacterial cells. It transferor specific reaction in bacterial cells on cytosine groups which results biochemical changes in mechanism of synthesis of DNA results death of cells. At low dose STZ interferes with cellular reproduction than prevention of synthesis of DNA or inhibitions of activity of enzymes involved in DNA synthesis[14-15]. STZ also blocks the entry of cells in cell division (mitosis) but it is not clear at which specific phase of division. Different doses of *Abies pindrow Royle* leaf extract (150, 300 and 600. mg/kg) were administered in rats for 28 successive days. On day 7th, 14th, 21th and 28thafter 15 min of drug administration body weight, changes in body weight of both adult and non-adult diabetic rats. varied. In non-adult diabetic rodent's loss of weight is not significantly as they are at growing stage and a slight increase in body weight is observed[19]. Administration of *Abies pindrow Royle* leaf extract for 28 successive days significantly produced anti-diabetic effect as indicated by a meaningful demotion in tail flick response time in hot water tail flick test and foot withdrawal latency in Eddy hot plate test in comparison of diabetic control were recorded. All treated groups were showed non-significant elevation in total body weight[6,20]. Drug administered groups expressed a significant reduction in cholesterol, VLDL, LDL, triglyceroids, creatinine levels as compared to vehicle group. Drug administered group expressed a significant elevation in HDL level as compare to vehicle were recorded[6,23]. Diabetic group administered glibenclamide (10 mg/kg) expressed anti-diabetic effect as expressed by a meaningful elevation in weight in comparison to vehicle group. In diabetes decrease anti-nociceptive effect because in diabetic neuropathy cause neuro degeneration due to loss of pain perception. Oxidative stress is also cause nerve deficiency in diabetic rats. Endoneurial hypoxia cause morphological and functional abnormality in nervous system and decrease nerve blood flow. Central and peripheral nervous system affected in hyperglycaemia due to enhancement of free radicals. In diabetes neuropathy pathogenesis some evidence found due free radicals formed in sorbitol pathway related to glucose metabolism. Antioxidant enzymes protect cells against reactive oxygen species such as exogenous and endogenous toxic compounds. Glucose oxidation and lipid peroxidation processes in diabetic state elevated level of toxic oxidants. Free radicle mediated toxicity increased in streptozotocin (STZ) induced diabetes in rats[21-22]. Direct toxicity increased in primary afferent fibers, reduced toxicity on opioidergic inhibitory systems, glutamate release increased, GABAergic inhibitory system activity reduced, altered the activity on both responsiveness of the dopaminergic system and dopaminergic receptors sensitivity (possibly through endogenous enkephalinergic system) and in diabetic rat's modulation of nociception. Metformin treated diabetic rats were significantly elevation in body weight of female rats due to increase of tissue protein[16]. This is supported by literature. In saffron treated diabetic rats also improve their body weight due to increment in tissue proteins. The degradation of the catabolism process of structural proteins cause decrease in body weight of rats. The catabolism process is occurring is due to energy metabolism or unavailability of carbohydrate ETGF (200 mg/kg) improved beta cell

functions lead to decrease level of blood glucose. This is supported by literature. DM (induced by STZ) blood glucose level decreased significantly after administration of wheatgrass and effect of wheatgrass is almost equal to glibenclamide standard drug this is supported by literature. The level of phospholipids decreased by treatments with extract of *E. floccosa* leaf (ethanolic extract) and glibenclamide (standard drug). Deficiency of insulin secretion inactivate lipoprotein lipase enzyme[18]. The conversion of free fatty acids by liver through lipoprotein lipase enzyme[17,20]. The discharge of cholesterol and phospholipids in blood which increase phospholipids level[16,23]. On administration of *Abies pindrow Royle* plant leaf extract in diabetic rats the levels of high density lipoproteins were found to be normal. Serum lipid profile usually rises in diabetic rats as compared to treated rats. After administration of *Abies pindrow Royle* plant leaf extract (150mg/kg) were significantly decreased DM treated rodents and HDL level rise significantly in treated diabetic animals. The elevation of blood serum levels increased hypertriglyceridemia and hypercholesteromia were caused in diabetic rats. Treatment with *Abies pindrow Royle* leaf extract (300 mg. /kg, p.o.) about. 28 sucessive days considerably produced anti-diabetic effect as indicated by a meaningful elevation in weight as compared to vehicle group and also as compare to standard drug treated rats. A non-meaningful increase in protein level as compared to standard group is expressed. Treatment with *Abies pindrow Royle* leaf extract (600 mg/kg, p.o.) for 28 consecutive days, considerably produced. anti-diabetic effect as indicated by a meaningful elevation in weight in comparison to vehicle group and also as compare to standard drug treated rats. A meaningful reduction in TG, TC, creatinine and VLDL in comparison of control rats (diabetic) was observed.

CONCLUSION

In animal models of anti - nociceptive *Abies pindrow Royle* leaf extract. (150, 300 and 600 mg. /kg) produced anti-analgesic effect. And the *Abies pindrow Royle* leaf extract (300 mg/kg) dose have anti-analgesic effect greater than other treatments of plant extract. All treatments of plant extract produce significant anti-diabetic activity as compare to diabetic control group. The 300. mg/kg. dose of plant extract was found to be most potent dose in this study. The plant extract. (300 mg/kg) dose show comparatively equal anti-diabetic effect as compare to standard drug treated rats. The anti-diabetic effect of plant can be due to elevation in activity of glucokinase enzyme present in pancreas.

ACKNOWLEDGEMENT-: The authors express their heartfelt gratitude to the Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, Haryana, for providing the necessary support, guidance, and facilities to carry out this work successfully.

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