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# EVALUATION OF ANTI-DIABETIC ACTIVITY OF LEAVES OF *ACTINODAPHNE HOOKERI* MEISSN.

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**Keywords:**

*Actinodaphne hookeri*, Anti-diabetic, *α*-glucosidase, Streptozotocin

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**ABSTRACT: Background:** *Actinodaphne hookeri* Meissn. (Family Lauraceae), known as Pisa, is a moderate evergreen tree, found commonly in Chattisgarh, Western Ghats, Maharashtra, Karnataka, Orissa, Sikkim and North-Eastern parts of India. The leaves have been traditionally used in the treatment of diabetes and urinary disorders. The leaves were also known to possess potent antioxidant activity. **Objective:** Aim of the study was to investigate the *in-vitro and in-vivo* anti-diabetic activity of different extracts of leaves of *Actinodaphane hookeri*. **Materials and Methods:** The leaves of *Actinodaphane hookeri* were extracted successively with petroleum ether (AHP), chloroform (AHC), methanol (AHM) and water (AHA). Also, the leaves were extracted separately with alcohol (AHT). Extracts were subjected to phytochemical investigation and evaluated for its *in-vitro* anti-diabetic activity by *α*-glucosidase inhibitory activity, and *in-vivo* anti-diabetic activity was carried out in STZ-induced diabetic rats at two dose levels *viz*. 300 and 500 mg/kg doses for 21 days. **Results:** Phytochemical screening showed the presence of sterols, triterpenes, alkaloids, flavonoids, tannins, lactones and mucilages. The *in-vitro α*-glucosidase inhibitory activity was highest in AHC with an $IC_{50}$ value of 309.04 ± 0.02 µg/ml and lowest in AHA with an $IC_{50}$ value of 812.86 ± 0.03 µg/ml. Significant decrease in blood sugar, gain in body weight, decrease in TCH, TG, LDL and VLDL and increase in HDL levels were observed. Potent activity was observed in AHC followed by AHP, AHT, AHM and AHA. Histopathological investigation showed pancreatic *β*-cell protection. **Conclusion:** The findings suggest that *Actinodaphne hookeri* Meissn. leaves have potent anti-diabetic activity.

**INTRODUCTION:** Diabetes Mellitus (DM) is known to Indians from Vedic period onwards by the name Asrava (Prameha, derived from the Sanskrit words mih sechane meaning 'watering' that means dilution of everything in the body.

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Diabetes is also known as Madhumeha in Ayurveda [1]. The high blood sugar produces the classic symptoms of polyuria, polydipsia and polyphagia [2].

There are two types of diabetes, namely, Type I and Type II [3]. Type I diabetes accounts for 5 to 10% of all diagnosed cases of diabetes. Type II diabetes or non-insulin - dependent diabetes mellitus (NIDDM) or adult-onset diabetes, accounts for about 90 to 95% of all diagnosed cases of diabetes. It usually begins as insulin resistance, a disorder in which the cells do not use insulin

properly, and as the need for insulin rises, the pancreas gradually loses its ability to produce it. This type of diabetes is associated with older age, obesity, family history of diabetes, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity [4]. Here, the treatment includes medicines, diets, and physical training. According to WHO, the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 in the world [5]. Indians are genetically more susceptible to diabetes and the WHO predicts the number of diabetic persons in India would go up to 74 million by 2025 [6].

Oral hypoglycemic agents (OHA) are widely used in the treatment, but they are known to have adverse effects [7]. Despite the impressive advances in health sciences and medical care, many patients are using alternative therapies alone or complementary to the prescribed medication. Traditional plant remedies or herbal formulations exist from ancient times and are still widely used, despite all the controversy concerning their efficacy and safety [8, 43] to treat hyperglycemic conditions all over the world. Many ethnobotanical surveys on medicinal plants used by the local population as anti-diabetic have been performed and a variety of compounds like alkaloids, glycosides, terpenes, flavonoids, *etc*. have been isolated, but further studies need to be done so these 'leads' to develop into clinically useful medicines.

*Actinodaphne hookeri* Meissn. (Lauraceae family) is a moderate sized tree or a shrub commonly called as Pisa, usually up to 6 m in height, found in the evergreen forests of Eastern and Western Ghats, Karnataka, Orissa, Sikkim and North-East India.[9] Traditionally the leaves are used in the treatment of diabetes and urinary disorders [10]. 70% hydroalcoholic extract of *A. hookeri* leaves display potent antioxidant properties, supporting the ethnomedical use given to this plant to treat diabetes and urinary disorder [24]. The ethanol and the chloroform extract of the leaves were also found to have significant blood glucose lowering effect on alloxan-induced diabetic rats [25, 26, 27, 28].

But due to reports stating an unpredictable onset of action of alloxan in inducing consistent diabetes and causing nephrotoxicity in experimental animals [75], an approach is taken to evaluate the hypo-glycaemic effect of various extracts of the plant leaves in streptozotocin-induced diabetes in this study.

## MATERIALS AND METHODS:

**Equipments, Chemicals and Reagents:** Heating Mantle (Sigma instruments), Hot Air Oven (Dolphin), Rotary Evaporator (Hahn Shin Scientific Co.), Semi Autoanalyser (Merck Microlabs), Soxhlet Apparatus, Glassware (Borosil), Water Bath (Servewell Instruments Pvt. Ltd., Bangalore), Binocular Microscope (SUSWOX, OPTIK), Trinocular microscope (Coslab, Techno-Scientific product), Glucometer (True balance), Digital Camera (Olympus FE-46), Colorimeter, Digital pH meter (Systronics), Digital Balance (Shimadzu)

Total cholesterol kit, triglyceride kit, total protein kit, glucose (Merck, Mumbai), streptozotocin (Sigma, Bangalore), glucose kit (Erba, Himachal Pradesh), $\alpha$ - glucosidase (SRL), acarbose (Biocon), glibenclamide (Sunpharma).

**Collection of Plant Material:** *A. hookeri* leaves were collected in July 2011, from Jog falls near a town called Sirsi of Karnataka State, India. The plant was authenticated by Dr. M. N. Naganandini, Assistant Professor, Department of Pharmacognosy, JSS College of Pharmacy, Mysore.

**Preparation of Extract:** [29, 30] Shade-dried and powdered leaves of *Actinodaphne hookeri* Meissn. (1 kg) were extracted by successive solvent extraction method using petroleum ether, chloroform, methanol, and water. Total extract was prepared using absolute alcohol. The extracts were concentrated under reduced pressure using flash rotary evaporator and dried on a water bath and then in the desiccator and preserved in the desiccator till use. The color and consistency were observed, and the percentage yield for each extract was calculated and given in **Table 1**.

**Preliminary Phytochemical Screening:** Preliminary phytochemical screening was carried out for a pet. ether (AHP), chloroform (AHC), methanol (AHM), aqueous (AHA) and total extracts (AHT) of *Actinodaphne hookeri* leaves to know the nature of chemical constituents present in it. Test for alkaloids, carbohydrates, flavonoids, lactones, saponins, tannins, sterols, triterpenes, and

mucilages were carried out using standardized procedures [31-33]. The results are given in **Table 2**.

### *In-vitro* Anti-Diabetic Activity [34]:

### *α*-glucosidase Inhibitory Activity:

**Procedure:** The inhibitory activity was determined by incubating 1 ml of starch substrate solution (2% w/v sucrose) and 1 ml of various concentrations of standard and plant extracts (100 µg/ml - 1000 µg/ml) for 5 min at 37 °C. The reaction was initiated by adding 1 ml of *α*-glucosidase enzyme (1 U/ml) followed by incubation for 10 min at 37 °C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase-peroxidase method [36]. The results are illustrated in **Graph 1**.

### *In-vivo* Anti-Diabetic Activity:

**Animals:** Anti-diabetic activity was carried out using 3 months old healthy Wistar albino rats of either sex weighing between 150- 250 g. They were procured from Central Animal Facility, JSS Academy of Higher Education and Research, Mysuru. The animal care and handling were carried out by the guidelines issued by the IAEC, JSS College of Pharmacy, Mysore, Karnataka. Wistar Albino rats were acclimatized to the experimental room for one week before the experiment. They were maintained under controlled temperature (23 ± 2 °C), controlled humidity (50 ± 5%), and 12:12 h light and dark cycle. Animals were caged in sterile polypropylene cages containing sterile paddy husk as bedding material with a maximum of two animals in each cage.

The rats were fed with standard food pellets and water *ad libitum*. All the experiments on animal were conducted according to protocols that were approved by the Institutional Animal Ethics Committee/IAEC and after obtaining ethical committee clearance from Institutional Animal Ethics Committee of JSS College of Pharmacy, Mysore no. IAEC/JSSMC/101/2011. Acute toxicity studies were carried out using Wistar Albino rats.

**Acute Toxicity Studies:** Acute toxicity studies were conducted according to OECD guidelines number 420. 5000 mg/kg body weight was found as the maximum tolerable dose (MTD) as the animals did not reveal any drug-related observable changes *viz.* behavioral, neuronal and autonomic, and did not cause mortality.

**Induction of Diabetes by Streptozotocin (STZ) Method: Selection of Doses:** The doses selected for the extracts were about $1/10^{th,}$ and $1/20^{th}$ of the maximum tolerated dose found from acute toxicity studies. They were administered once daily by the oral route. 300 mg/kg body wt. and 500 mg/kg body wt. doses were selected for all extracts to carry out the study. The dose of the standard drug, glibenclamide selected was 10 mg/kg body weight of rat [37].

**Animal Grouping:** The selected animals of 90 days old, weighing between 150-250 g were fasted overnight and administered with STZ 40 mg/kg IP. Fasting blood sugar levels were determined on the $14^{th}$ day after administering STZ to confirm stable hyperglycemia. The blood glucose level was more than 250 mg/dl.

The diabetic rats after confirmation of stable hyperglycemia were divided into different groups of 6 rats each. That day was considered as the 0 days. The effect of extracts was studied on diabetic rats for 21 days. The animals were grouped as follows - Normal (food and water *ad libitum)*, Standard (standard drug Glibenclamide), Diabetic Control (vehicle 1% gum acacia), AHP 300 (300 mg/kg dose of petroleum ether extract), AHP 500 (500 mg/kg dose of petroleum ether extract), AHC 300 (300 mg/kg dose of chloroform extract), AHC 500 (500 mg/kg dose of chloroform extract), AHM 300 (300 mg/kg dose of methanol extract), AHM 500 (500 mg/kg dose of methanol extract), AHA 300 (300 mg/kg dose of aqueous extract), AHA 500 (500 mg/kg dose of aqueous extract), AHT 300 (300 mg/kg dose of total extract), AHT 500 (500 mg/kg dose of total extract).

Blood samples were withdrawn on day 7, 14 and 21 by tail vein method. The fasting blood sugar levels were determined using the glucose strips. On the $21^{st}$ day, animals were sacrificed, and the following parameters were monitored.

Blood sugar level was determined by using TRUE Balance blood glucose meter and TRUE Balance glucose strips by collecting blood from tail vein. The results are given in **Table 3** and illustrated in **Graph 2a** and **b**.

Body weight of each animal was checked on the 0th and 21st day. The results are given in **Table 4** and illustrated in **Graph 3a** and **b**.

Lipid profile was determined after the rats were anesthetized using ether chamber and blood was withdrawn from retro-orbital plexus by capillary method. The serum triglycerides and serum cholesterol levels were determined in the serum of the collected blood [37]. Estimation of total Cholesterol was done by CHOD-POD method [38]. The results are given in **Table 5** and illustrated in **Graph 4a** and **b**. Estimation of serum triglycerides was done by GPO-POD method [39]. The results are given in Table 5 and illustrated in **Graph 5a** and **b**. Estimation of HDL cholesterol level was done by Phosphotungstate method [40]. The absorbance was read at 510 nm against the reagent (blank) in Autoanalyzer. LDL and VLDL cholesterol were estimated by using Friedwald's formula [41]. The results for HDL, LDL and VLDL levels are given in **Table 6** and illustrated in **Graph 6a** and **b**; **7a** and **b**; **8a** and **b** respectively. Serum creatinine level was estimated using Jaffe's method [42]. The effect of 300 mg/kg and 500 mg/kg doses of all extracts on the serum creatinine level was evaluated on the 21st day. The results are given in **Table 7** and illustrated in **Graph 9a** and **b**.

**Histopathological Studies:** On day 21, when the animals were sacrificed, the pancreas of animals from all the groups were excised and stored in 10% formalin after washing with normal saline. Histopathological examination included H and E staining of pancreatic tissue to observe any morphological changes in the cells present. The tissue was washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial sections of 5 µm thickness were cut using a rotary microtome. The sections were then deparaffinized with xylene and hydrated in descending grades of alcohol. The slides were then transferred to hematoxylin for 10 min.

This was followed by rinsing with water. These were examined and later counterstained with eosin, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted. Slides were observed under light microscope, and the effects of drug treatment on the beta cells were analyzed.

**Statistical Analysis:** The values are expressed as mean ± standard error of the mean (SEM) of the indicated number of experiments/animals. Statistical analysis was performed using GraphPad Prism version 5.04 by one way one way ANOVA followed by post hoc Tukey's multiple comparison tests. A value of $p < 0.05$ was considered significant. Graphs were prepared by GraphPad Prism version 5.04 software.

## RESULTS:

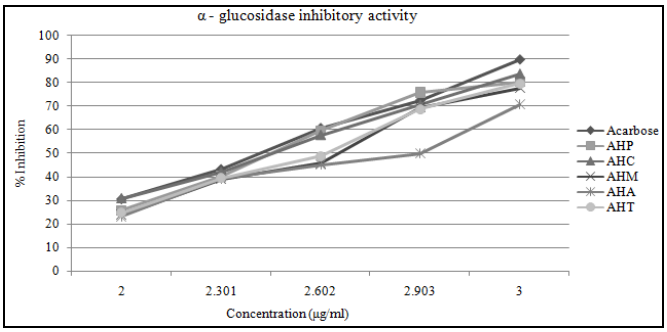
**Preliminary Phytochemical Screening:** Preliminary phytochemical screening showed the presence of sterols and triterpenes in AHP; triterpenes and alkaloids in AHC; flavonoids, tannins, lactones and carbohydrates in AHM; carbohydrates and mucilages in AHW; sterols, triterpenes, alkaloids, flavonoids, tannins, lactones, carbohydrates and mucilages in AHT.

**TABLE 1: PERCENTAGE YIELD OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI***

| Extracts | Colour and Consistency | Percentage yield (% w/w) |
|---|---|---|
| AHP | Dark green (sticky solid) | 5 |
| AHC | Dark green (sticky semisolid) | 8 |
| AHM | Reddish brown (dry solid) | 12 |
| AHA | Dark brown (dry solid) | 4 |
| AHT | Blackish green (dry solid) | 20 |

**TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF *A. HOOKERI* LEAVES**

| Chemical Constituents | AHP | AHC | AHM | AHA | AHT |
|---|---|---|---|---|---|
| Alkaloids | - | + | - | - | + |
| Carbohydrates | - | - | + | + | + |
| Flavonoids | - | - | + | - | + |
| Lactones | - | - | + | - | + |
| Saponins | - | - | - | - | - |
| Tannins | - | - | + | - | + |
| Sterols | + | - | - | - | + |
| Triterpenes | + | + | - | - | + |
| Mucilages | - | - | - | + | + |




**GRAPH 1: *IN-VITRO* *α*-GLUCOSIDASE INHIBITORY ACTIVITY OF VARIOUS EXTRACTS OF *A. HOOKERI***

***In-vitro* *α*-glucosidase Inhibitory Activity:** Acarbose showed the least $IC_{50}$ value 232.87 ± 0.03

µg/ml and the $IC_{50}$ value of AHC, AHP, AHT, AHM and AHA were found to be 309.04 ± 0.02, 363.11 ± 0.02, 588.97 ± 0.06, 794.45 ± 0.05, 812.86 ± 0.03 µg/ml respectively.

### *In-vivo* Anti-Diabetic Activity:

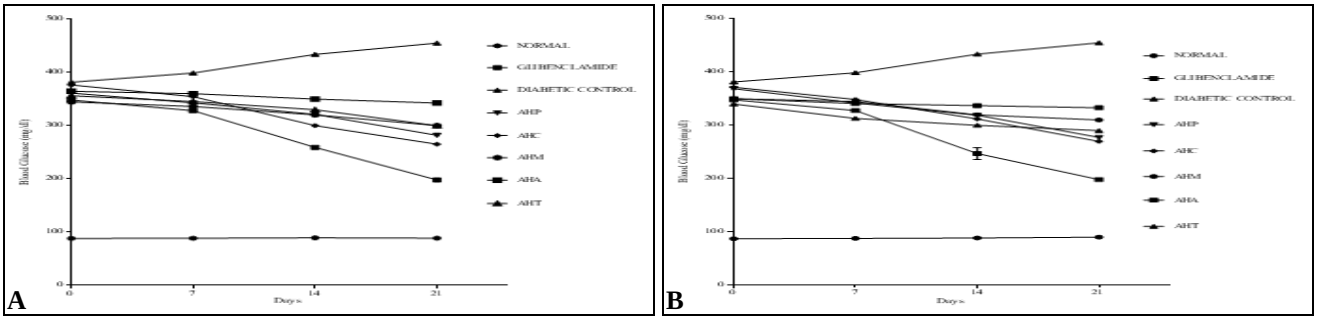
**Evaluation of Blood Sugar Level:** All extracts showed significant results ($p<0.05$) when compared to the diabetic control group. The most potent hypoglycemic effect was observed in the groups treated with 300 and 500 mg/kg dose of AHC and AHP, followed by AHT and AHM. Last activity was observed in diabetic animals treated with AHA.

**TABLE 3: EFFECT OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON BLOOD GLUCOSE LEVEL IN RATS**

| Groups | Blood glucose level (mg/dl) | | | |
|---|---|---|---|---|
| | 0 day | 7th day | 14th day | 21st day |
| Normal | 86.83 ± 1.58 | 87.50 ± 1.71 | 88.17 ± 1.58 | 89.67 ± 0.80[a] |
| Glibenclamide | 348.00 ± 1.57 | 328.17 ± 1.01 | 259.00 ± 0.97 | 198.00 ± 1.13[b] |
| Diabetic Control | 381.50 ± 0.76 | 398.83 ± 0.87 | 433.83 ± 0.95 | 455.17 ± 0.48[c] |
| AHP 300 mg/kg | 361.17 ± 0.79 | 342.83 ± 0.87 | 321.17 ± 0.60 | 281.83 ± 0.79[abc] |
| AHC 300 mg/kg | 376.50 ± 0.43 | 354.50 ± 0.43 | 300.17 ± 0.48 | 265.17 ± 0.48[abc] |
| AHM 300 mg/kg | 344.50 ± 0.43 | 335.83 ± 0.48 | 320.33 ± 0.49 | 300.17 ± 0.48[abc] |
| AHA 300 mg/kg | 364.50 ± 0.43 | 360.17 ± 0.48 | 349.83 ± 0.31 | 342.50 ± 0.43[ab] |
| AHT 300 mg/kg | 356.00 ± 0.37 | 345.17 ± 0.48 | 330.00 ± 0.58 | 300.00 ± 0.58[ab] |
| AHP 500 mg/kg | 368.83 ± 0.31 | 342.00 ± 0.37 | 319.00 ± 0.37 | 277.17 ± 0.48[abc] |
| AHC 500 mg/kg | 371.50 ± 0.43 | 348.67 ± 0.49 | 311.83 ± 0.31 | 269.67 ± 0.42[abc] |
| AHM 500 mg/kg | 350.00 ± 0.37 | 344.83 ± 0.31 | 319.67 ± 0.42 | 310.00 ± 0.37[abc] |
| AHA 500 mg/kg | 350.00 ± 0.37 | 341.00 ± 0.37 | 337.00 ± 0.37 | 333.00 ± 0.37[ab] |
| AHT 500 mg/kg | 340.00 ± 0.37 | 313.00 ± 0.37 | 300.00 ± 0.58 | 290.00 ± 0.37[abc] |

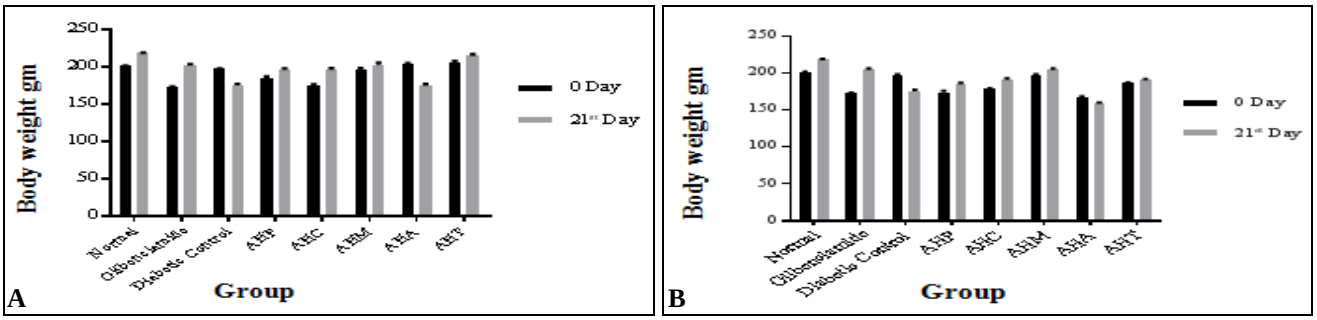
p = Projected value

All values are expressed as Mean ± SEM, n=6, [a]$p<0.05$ compared to Normal, [b]$p>0.05$ compared to Standard and [c]$p< 0.05$ compared to Control. All the data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.




**GRAPH 2: EFFECT OF 300 mg/kg (A) AND 500 mg/kg (B) BODY WEIGHT DOSE OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE BLOOD GLUCOSE LEVEL IN RATS**

**Evaluation of Body Weight:** Normal animals did not show much variation in their body weight. Animals treated with standard Glibenclamide showed significant weight gain on the 21st day. The diabetic control group showed a decrease in the body weight. Animals treated with 300 mg/kg and 500 mg/kg dose of AHP, AHC, AHM, and AHT showed weight gain on the final day, but in case of AHA, body weight was reduced.




**GRAPH 3: EFFECT OF 300 mg/kg (A) AND 500 mg/kg (B) DOSE OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE BODY WEIGHT OF DIABETIC RATS**

**TABLE 4: EFFECT OF 300 mg/kg AND 500 mg/kg DOSE OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE BODY WEIGHT OF DIABETIC RATS**

| Groups | 0 day (in g) | 21st day (in g) |
|---|---|---|
| Normal | 201.33 ± 0.49 | 218.83 ± 0.48 |
| Glibenclamide | 172.67 ± 1.23 | 202.67 ± 1.23 |
| Diabetic Control | 197.67 ± 0.61 | 175.67 ± 1.89 |
| AHP 300 mg/kg | 185.00 ± 2.23 | 196.00 ± 1.93 |
| AHC 300 mg/kg | 174.67 ± 2.11 | 196.00 ± 2.03 |
| AHM 300 mg/kg | 196.67 ± 2.11 | 203.00 ± 3.13 |
| AHA 300 mg/kg | 203.33 ± 2.11 | 175.00 ± 2.24 |
| AHT 300 mg/kg | 207.00 ± 1.34 | 215.00 ± 2.24 |
| AHP 500 mg/kg | 174.00 ± 1.79 | 185.00 ± 2.24 |
| AHC 500 mg/kg | 178.67 ± 0.67 | 191.00 ± 1.84 |
| AHM 500 mg/kg | 197.67 ± 0.61 | 205.33 ± 0.99 |
| AHA 500 mg/kg | 167.67 ± 0.80 | 158.50 ± 1.80 |
| AHT 500 mg/kg | 187.00 ± 0.68 | 190.67 ± 1.61 |

Data expressed as mean ± SD, n=6.

**Evaluation of Lipid Parameters:**

**Total Cholesterol Level:** All groups showed lowering of TCH level. 300 and 500 mg/kg dose of AHC, AHM, and AHT showed a significant decrease ($p<0.05$) when compared to diabetic control group. The results were not significant in the case of 500 mg/kg of AHP and AHA when compared to diabetic control groups.

**Triglyceride Level:** When compared to diabetic control group, both doses of AHP, AHC, AHA and AHT showed a significant decrease ($p<0.05$). Both doses of all extracts, except AHA, showed a significant increase ($p<0.05$) when compared to Glibenclamide group but was not significant ($p>0.05$) in case of AHT.

**HDL-Cholesterol Level:** When compared to diabetic control group both doses of AHP, AHC, AHM, and AHA showed a significant increase ($p<0.05$). Both doses of AHP and AHA showed a significant increase ($p<0.05$) when compared to the Normal group but the results were not significant ($p>0.05$) in case of AHC and AHM.

**LDL-Cholesterol Level:** When compared to diabetic control group both doses of all extracts showed a significant decrease ($p<0.05$) but AHA was not significant ($p>0.05$). AHA showed a significant increase compared to the normal group.

**VLDL-Cholesterol Level:** All groups showed a decrease in VLDL-Cholesterol level compared to diabetic control group out of which AHM was not-significant ($p>0.05$).

**TABLE 5: EFFECT OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE CHOLESTEROL AND TRIGLYCERIDE LEVELS IN DIABETIC RATS**

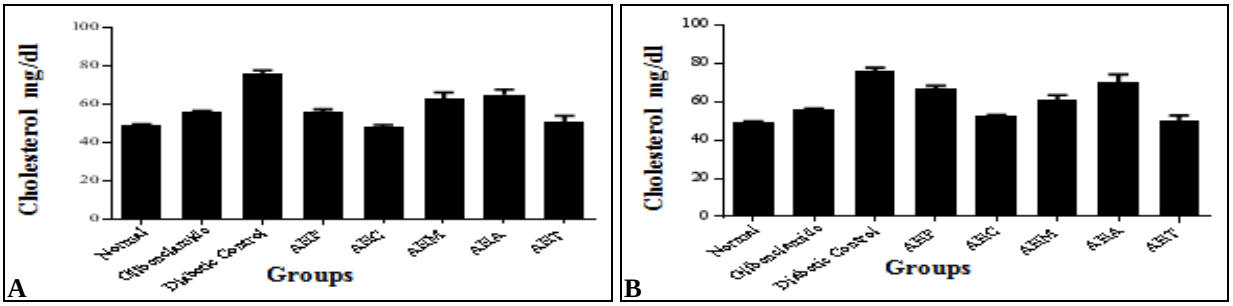
| Groups | Cholesterol (mg/dl) | Triglyceride (mg/dl) |
|---|---|---|
| Normal | $49.00 \pm 0.73^{a}$ | $95.83 \pm 0.31^{a}$ |
| Glibenclamide | $55.83 \pm 0.70^{b}$ | $90.67 \pm 0.33^{b}$ |
| Diabetic Control | $76.00 \pm 1.81^{ca}$ | $138.67 \pm 0.96^{ca}$ |
| AHP 300 mg/kg | $55.83 \pm 1.52^{bc}$ | $105.50 \pm 3.24^{c}$ |
| AHC 300 mg/kg | $47.83 \pm 1.42^{bc}$ | $122.17 \pm 3.84^{ac}$ |
| AHM 300 mg/kg | $63.00 \pm 3.26^{abc}$ | $130.00 \pm 3.58^{a}$ |
| AHA 300 mg/kg | $65.00 \pm 2.81^{abc}$ | $40.50 \pm 4.26^{ac}$ |
| AHT 300 mg/kg | $50.50 \pm 3.52^{bc}$ | $94.17 \pm 5.01^{bc}$ |
| AHP 500 mg/kg | $66.83 \pm 1.60^{a}$ | $94.17 \pm 5.21^{bc}$ |
| AHC 500 mg/kg | $52.50 \pm 0.67^{bc}$ | $129.67 \pm 5.40^{a}$ |
| AHM 500 mg/kg | $61.00 \pm 2.57^{abc}$ | $141.50 \pm 10.14^{a}$ |
| AHA 500 mg/kg | $70.50 \pm 3.80^{a}$ | $41.00 \pm 4.47^{ac}$ |
| AHT 500 mg/kg | $50.00 \pm 2.99^{bc}$ | $92.17 \pm 9.22^{bc}$ |

All values are expressed as Mean ± SEM, n=6, $^{a}p<0.05$ compared to Normal, $^{b}p>0.05$ compared to Standard, $^{c}p<0.05$ compared to Control. All the data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.

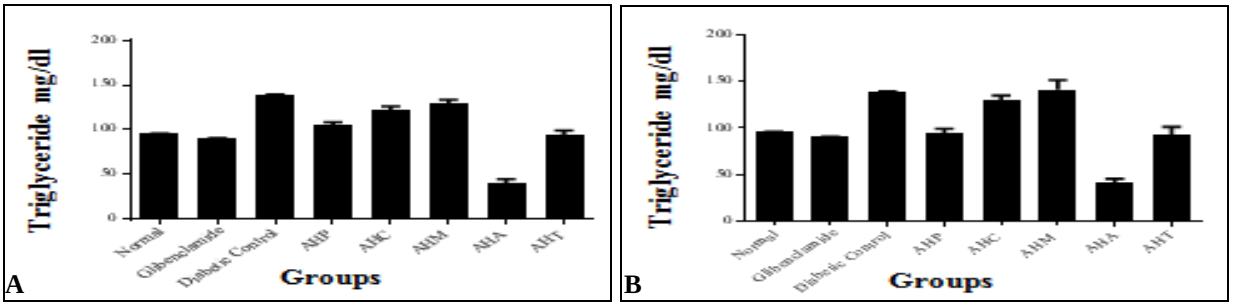
**TABLE 6: EFFECT OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE HDL, LDL AND VLDL-CHOLESTEROL LEVELS IN DIABETIC RATS**

| Groups | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) |
|---|---|---|---|
| Normal | $14.83 \pm 0.60^{a}$ | $15.67 \pm 0.21^{a}$ | $19.17 \pm 0.06^{a}$ |
| Glibenclamide | $16.50 \pm 0.22^{ba}$ | $21.20 \pm 0.76^{b}$ | $18.13 \pm 0.07^{b}$ |
| Diabetic Control | $9.83 \pm 0.54^{ca}$ | $38.43 \pm 1.84^{ca}$ | $27.73 \pm 0.19^{ca}$ |
| AHP 300 mg/kg | $24.67 \pm 1.15^{ac}$ | $10.07 \pm 2.40^{c}$ | $21.10 \pm 0.65^{c}$ |
| AHC 300 mg/kg | $14.50 \pm 1.75^{bc}$ | $8.90 \pm 1.78^{c}$ | $24.43 \pm 0.77^{ac}$ |
| AHM 300 mg/kg | $15.00 \pm 0.82^{bc}$ | $22.00 \pm 3.31^{bc}$ | $26.00 \pm 0.72^{a}$ |
| AHA 300 mg/kg | $27.33 \pm 1.48^{ac}$ | $29.57 \pm 2.78^{ab}$ | $8.10 \pm 0.85^{ac}$ |
| AHT 300 mg/kg | $14.00 \pm 0.73^{b}$ | $17.67 \pm 2.72^{bc}$ | $18.83 \pm 1.00^{bc}$ |
| AHP 500 mg/kg | $28.33 \pm 2.11^{ac}$ | $19.77 \pm 2.14^{bc}$ | $18.83 \pm 1.04^{bc}$ |
| AHC 500 mg/kg | $19.00 \pm 0.45^{bc}$ | $7.57 \pm 1.35^{c}$ | $25.93 \pm 1.08^{a}$ |
| AHM 500 mg/kg | $14.33 \pm 0.62^{b}$ | $18.37 \pm 3.22^{bc}$ | $28.30 \pm 2.03^{a}$ |
| AHA 500 mg/kg | $32.00 \pm 1.90^{ac}$ | $30.30 \pm 4.07^{ab}$ | $8.20 \pm 0.89^{ac}$ |
| AHT 500 mg/kg | $9.00 \pm 1.79^{a}$ | $22.57 \pm 3.15^{bc}$ | $18.43 \pm 1.84^{bc}$ |

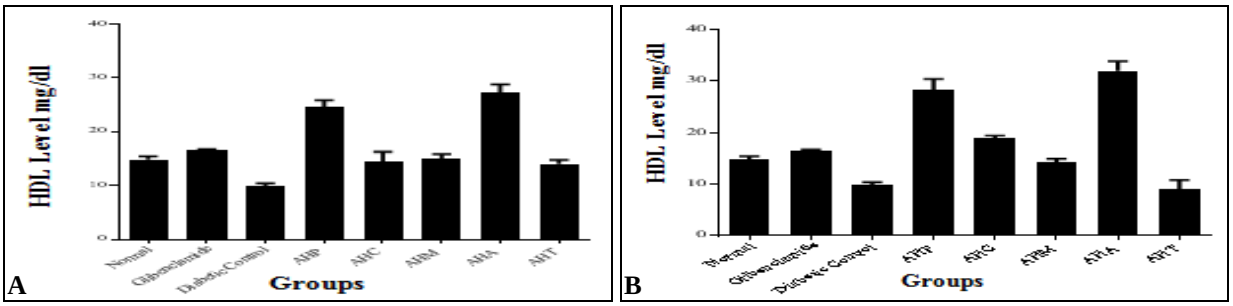
All values are expressed as Mean ± SEM, n=6, $^{a}p<0.05$ compared to Normal, $^{b}p>0.05$ compared to Standard, $^{c}p<0.05$ compared to Control. All the data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.



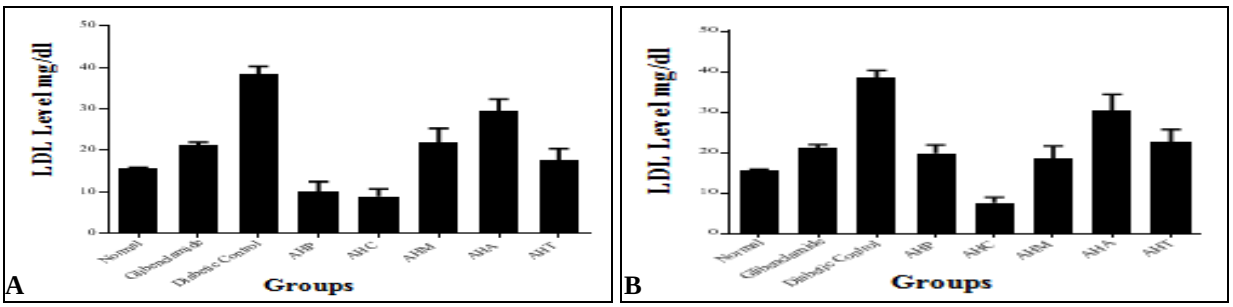

**GRAPH 4: EFFECT OF 300 mg/kg (A) AND 500 mg/kg (B) DOSE OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE TOTAL SERUM-CHOLESTEROL LEVEL IN DIABETIC RATS**



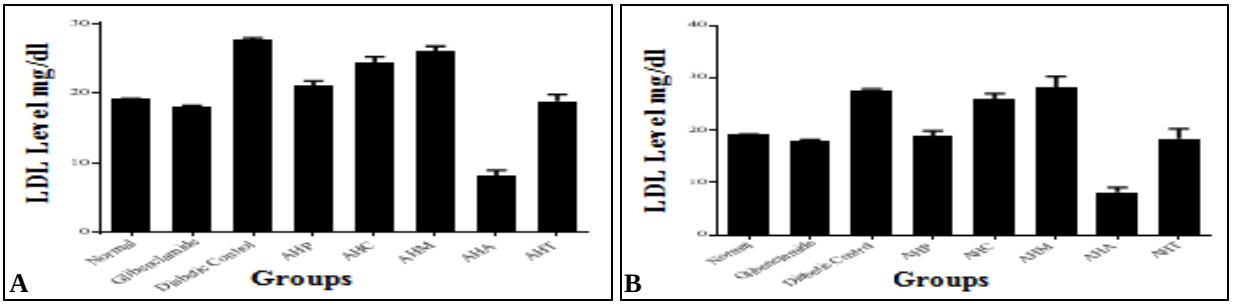

**GRAPH 5: EFFECT OF 300 mg/kg (A) AND 500 mg/kg (B) DOSE OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE SERUM TRIGLYCERIDE LEVEL IN DIABETIC RATS**




**GRAPH 6: EFFECT OF 300 mg/kg (A) AND 500 mg/kg (B) DOSE OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE HDL - CHOLESTEROL LEVEL IN DIABETIC RATS**

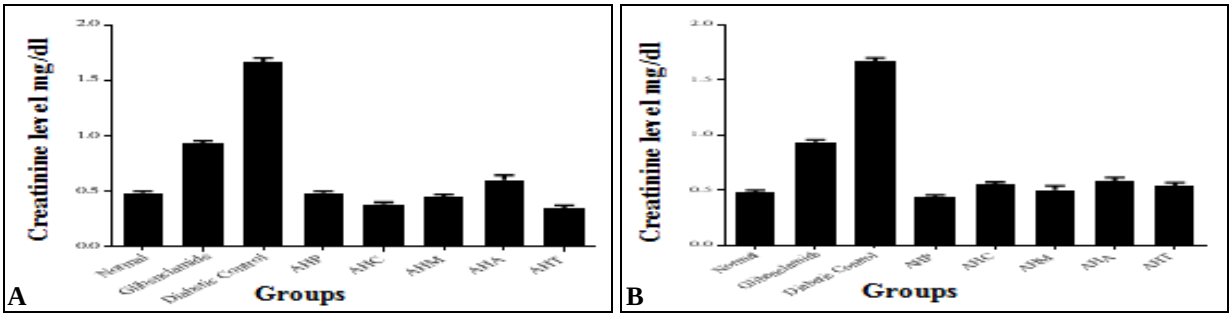



**GRAPH 7: EFFECT OF 300 mg/kg (A) AND 500 mg/kg (B) DOSE OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE LDL-CHOLESTEROL LEVEL IN DIABETIC RATS**




**GRAPH 8: EFFECT OF 300 mg/kg (A) AND 500 mg/kg (B) DOSE OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE VLDL-CHOLESTEROL LEVEL IN DIABETIC RATS**

**Evaluation of Serum Creatinine Level:** Significant variations were observed when compared to normal, Glibenclamide and diabetic control groups. The diabetic control group also showed a significant increase compared to Glibenclamide group. The test groups showed a significant decrease ($p<0.05$) compared to the diabetic control group. The levels were nearly close to the normal group.



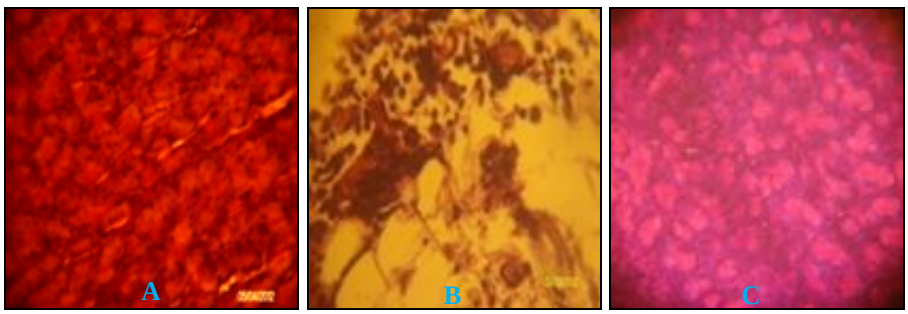

**GRAPH 9: EFFECT OF 300 mg/kg (A) AND 500 mg/kg (B) DOSE OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE SERUM CREATININE LEVEL IN DIABETIC RATS**

**TABLE 7: EFFECT OF VARIOUS EXTRACTS OF LEAVES OF *A. HOOKERI* ON THE SERUM CREATININE LEVEL IN DIABETIC RATS**

| Groups | Creatinine (mg/dl) |
|---|---|
| Normal | $0.48 \pm 0.02^{a}$ |
| Glibenclamide | $0.93 \pm 0.02^{ba}$ |
| Diabetic Control | $1.67 \pm 0.03^{cab}$ |
| AHP 300 mg/kg | $0.48 \pm 0.02^{bc}$ |
| AHC 300 mg/kg | $0.38 \pm 0.02^{bc}$ |
| AHM 300 mg/kg | $0.45 \pm 0.02^{bc}$ |
| AHA 300 mg/kg | $0.60 \pm 0.05^{bc}$ |
| AHT 300 mg/kg | $0.35 \pm 0.02^{abc}$ |
| AHP 500 mg/kg | $0.43 \pm 0.02^{bc}$ |
| AHC 500 mg/kg | $0.55 \pm 0.02^{bc}$ |
| AHM 500 mg/kg | $0.50 \pm 0.04^{bc}$ |
| AHA 500 mg/kg | $0.58 \pm 0.03^{bc}$ |
| AHT 500 mg/kg | $0.53 \pm 0.03^{bc}$ |

All values are expressed as Mean ± SEM, n=6, $^{a}p<0.05$ compared to Normal, $^{b}p<0.05$ compared to Standard and $^{c}p<0.05$ compared to Control. All the data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.

**Histopathological Studies:** Histopathology of *β*-cells of rat pancreas was studied after the experimental period. **Fig. 10a**, the normal group, showed normal architecture with numerous compactly arranged cells. The diabetic control group, **Fig. 10b** showed degeneration and lytic changes of the cells. Few were shrunken, few were round/ovoid and showed inflammatory cellular infiltration with fibrosis. Treatment with Glibenclamide inhibited streptozotocin-induced shrinking of islets of langerhans, inflammatory cellular infiltration and enlarged pancreatic cells shown in **Fig. 10c**. Both doses of AHP were found to be effective on *β*-cells **Fig. 10d** and **e**. More prominent protection was observed on treatment with AHC on all the effects of STZ dose-dependently **Fig. 10f** and **g**. AHM did not show satisfactory results. Shrinking of cells and fibrosis was observed at moderate dose **Fig. 10h** but higher dose was little effective **Fig. 10i**. AHA was least effective in protection of the cells **Fig. 10j** and **k**. AHT showed the mild effect on the symptoms of STZ **Fig. 10l** and **m**. AHC increased the size of islets of langerhans, inhibited lymphocytic infiltration and vascular changes. The effect of AHC was highest followed by AHP, AHM, AHT, and AHA on the inhibitory effect of lysis and shrinking of *β*-cells.




**NORMAL** **DIABETIC CONTROL** **GLIBENCLAMIDE**

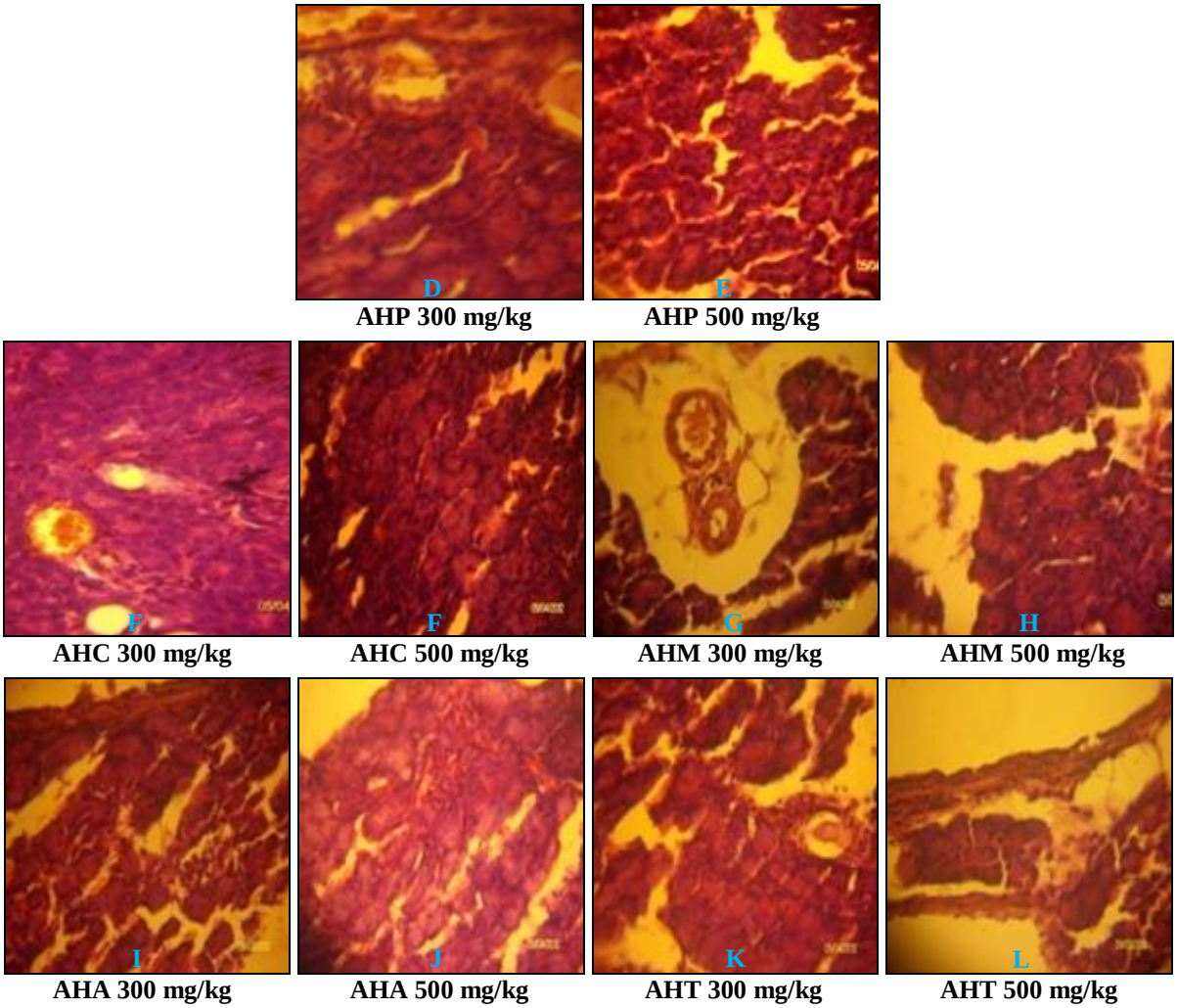



**FIG. 10: PHOTOMICROGRAPH OF ISLET OF LANGERHANS OF DIABETIC RATS OF VARIOUS GROUPS**

**DISCUSSION:** In the present study anti-diabetic activity of leaves of the plant *Actinodaphne hookeri* Meissn. was evaluated and was studied for its phytochemical nature. The leaves of the plant were extracted by successive solvent extraction method using petroleum ether, chloroform, methanol, water, and absolute alcohol and their percentage yield was calculated. The percentage yield of AHT was the highest (20% w/w). AHP showed low percentage yield (5% w/w) followed by AHC (8% w/w) and AHM (12% w/w). Cold maceration was carried out for aqueous extraction and showed the least percentage yield (4% w/w).

This difference in the % yield is due to the phytochemical nature of different extracts. *i.e.*, as phytochemical screening revealed that AHP contained only sterols and triterpenes; AHC contained triterpenes and alkaloids; AHM contained flavonoids, tannins, lactones, and carbohydrates; AHA contained only carbohydrates and mucilages; AHT contained all the above constituents *viz.* sterols, triterpenes, alkaloids, flavonoids, tannins, lactones, carbohydrates and mucilages.

The plant, *A. hookeri* has been documented as a therapeutic anti-diabetic agent in the traditional system of medicine [16]. Previously, pharmacognostical evaluation, [11-22] antioxidant activity [12-24] and alloxan-induced anti-diabetic activity [25-28] for this plant were reported. So, it is evident that plant is known to possess anti-diabetic activity. But alloxan was reported to show one major drawback *i.e.* reversibility of the induced diabetic state 20 days after IP administration [44]. Therefore, the STZ-induced NIDDM model was used for evaluating the anti-diabetic activity of various phytochemicals present in different extracts of the leaves of this plant. Also, attempt has been made to understand

the constituents responsible for its anti-diabetic activity.

The extracts were evaluated for *α*-glucosidase inhibitory activity by *in-vitro* method. *α*-glucosidase is an enzyme that breaks down starch and disaccharides into glucose. It hydrolyzes the terminal 1-4 linked α-glucose residues to release single α-glucose molecule [45]. *α*-glucosidase inhibitors are competitive, reversible inhibitors of pancreatic membrane-bound intestinal α-glucosidase hydrolase enzymes. They block the enzymatic degradation of complex carbohydrates in the small intestine. Thus, less carbohydrate is absorbed and delayed carbohydrate absorption takes place [35, 46]. Thus, these inhibitors help control glycemia by slowing down the digestion of complex carbohydrates in the intestine. Drugs belonging to this class only work in the intestine and enter the bloodstream in negligible amounts [47].

In the present study, acarbose was used as a working standard. From the results, it was observed that acarbose showed the most potent inhibitory activity with $IC_{50}$ of 232.87 ± 0.03 µg/ml. In extracts, AHC showed most potent inhibitory activity with $IC_{50}$ of 309.04 ± 0.02 µg/ml. The inhibitory activity might be due to the presence of triterpenes and alkaloids as shown by phytochemical tests. AHP also showed potent inhibitory activity with $IC_{50}$ of 363.11 ± 0.002 µg/ml which might be due to sterols, triterpenes, and alkaloids present in it. So the activity could be due to triterpenes and alkaloids. AHT showed $IC_{50}$ value 588.97 ± 0.06 µg/ml, and the inhibitory activity might be due to the combined effect of all the chemical constituents present in it. AHM showed less inhibitory activity with $IC_{50}$ of 794.45 ± 0.05 µg/ml. Sterols, triterpenes, and alkaloids were found to be absent in AHM. Though the *α*-glucosidase inhibitory activity is less in AHM, the extract has an activity. This activity may be due to flavonoids, tannins, and lactones. The lowest $IC_{50}$ value 812.86 ± 0.03 µg/ml was shown by AHA. It was found to contain only carbohydrates from preliminary phytochemical results. Sterols, triterpenes, alkaloids, flavonoids, tannins, saponins, glycosides, coumarins and anthraquinones have been reported to show *α*-glucosidase inhibitory activity [48, 49]. Therefore, the inhibitory activity could be possibly due to sterols, triterpenes, alkaloids, flavonoids and tannins and present in different extracts of *A. hookeri* leaves.

The fundamental mechanism underlying hyperglycemia in DM involved the overproduction (excessive hepatic glycogenolysis and gluconeo-genesis) and decreased utilization of glucose by the tissues [50]. Persistent hyperglycemia, the common characteristic of diabetes, can cause most diabetic complications. In all patients, treatment should aim over to lower blood glucose to or near normal levels [51]. In the present work, STZ-induced diabetic model was employed. In animals, diabetes can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as streptozotocin, alloxan, dithizone and anti-insulin serum. STZ is a naturally occurring nitrosourea product of *Streptomyces achromogenes* and is widely used to induce diabetes in experimental animals. The mechanisms by which STZ induces DM include stimulation of free radical generation [52] which may lead to selective destruction of pancreatic insulin secreting *β*-cells, which makes the cells less active [53] and leads to poor glucose utilization by tissues [54]. Usually, the intraperitoneal injection of a single dose (50 mg/kg b.w.) of it exerts toxicity on *β*-cells resulting in necrosis within 48-72 h and causes a permanent hyperglycemia [55]. The study reports are in good agreement with the reports documented that STZ-induced DM and insulin deficiency leads to increased blood glucose [56].

From the reports observed, animals treated with test extracts revealed a significant reduction in the blood sugar level when compared with diabetic control groups at the end of the experimental period. Both doses of AHC showed significant hypoglycemic activity compared to all other extracts. The significant decrease in blood sugar levels of diabetic rats may be comparable to that of the standard Glibenclamide and may be correlated with the *in-vitro* reports. The standard drug Glibenclamide has been used to treat diabetes, which stimulates insulin secretion from pancreatic *β*-cells [57]. The possible mechanism by which the extracts show significant hypoglycemia might be by the retained capacity of surviving *β*-cells to synthesize and secrete insulin [55] or by increasing the peripheral glucose uptake [57]. Chloroform extract showed significant $IC_{50}$ value near to

acarbose. This indicates that it might have anti-diabetic activity similar to acarbose.

Since, phytochemical investigation reports the presence of major active constituents *viz.* sterols, triterpenes, alkaloids, flavonoids, tannins and lactones in these extracts, these compounds may be responsible for the activity, which has also been previously reported [48-61]. Aqueous extract containing only carbohydrates and mucilages showed poor hypoglycemic activity. Also, its $IC_{50}$ value was the highest, which indicates the least inhibitory activity. So, probably these active constituents might be less responsible for the hypoglycemic effect.

Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting in diabetes [62]. Diabetic rats treated with test extracts *viz.* AHP, AHC, AHM, and AHT showed significant weight gain when compared to the diabetic control group. AHA showed weight loss by the end of 21 days.

In diabetic rats, a marked increase in Serum TG, LDL and VLDL levels and a decrease in HDL level were observed. These observations could be supported with reports documented earlier [63-65]. In this study, sample extracts have shown lowering of TG, TCH, LDL and VLDL levels and increase in HDL level compared to the control group. AHP, AHC, AHM, and AHT showed significant results. The results for AHA were not effective. Therefore, it can be said that the all major active constituents, *viz*. sterols, [66, 67] triterpenes, [68] alkaloids, [69] flavonoids [70] and tannins [68] present in the extracts have a role in the lipid metabolism. Catabolism of protein and nucleic acids results in the formation of non-protein nitrogenous compounds urea and creatinine. During DM, amino acids break down in the liver resulting in increased production of urea and creatinine [71]. The diabetic animals treated with test extracts showed a significant decrease in the reduction of creatinine.

The plant has also been proven to show potent antioxidant activity. The antioxidant property was due to flavonoids and phenolics that are present in the plant [12-24]. This can be correlated with anti-diabetic activity since free radicals and ROS have been claimed to play an important role in affecting health causing several diseases such as cancer, diabetes, aging, atherosclerosis, hypertension, heart attack and other degenerative diseases [72].

Flavonoids, phenols and lignin precursors are regarded to be beneficial antioxidants as they exhibit scavenging activity of harmful active oxygen species [73].

The effect of AHC containing triterpenes and alkaloids on lipid parameters appeared better followed by AHP containing sterols and triterpenes followed by AHM containing flavonoids, tannins, and lactones followed by AHT containing sterols, triterpenes, alkaloids, flavonoids, tannins, lactones, carbohydrates, and mucilages. The effect appeared low in case of AHA containing carbohydrates and mucilages. The observations appeared similar to those in case of lowering of blood sugar level and *α*-glucosidase inhibitory.

Histopathological observations support the hypoglycaemic reports. Administration of STZ brought about degenerative changes in the histopathology of pancreas when compared to normal animals. The protective effect of AHC was followed by AHP, AHT, AHM, and AHA. This shows that the changes initiated by STZ-induced diabetes were partially reversed by various extracts of AH.

So, it indicates that the anti-diabetic of *A.hookeri* was perhaps due to sterols, triterpenes, alkaloids, flavonoids and tannins and might be having multiple mechanisms *viz*. scavenging free radicals, ROS *etc*. [24]; competitive and reversible inhibition of pancreatic α-amylase and membrane-bound intestinal *α*-glucosidase enzymes; improving glucose tolerance by lowering basal and postprandial plasma glucose; by decreasing hepatic glucose production which decreases intestinal absorption of glucose; improving insulin sensitivity by increasing peripheral glucose uptake and utilization; potent agonist for the PPARγ receptors; interacting with $K^+$ ATP channel on pancreatic *β*-cells and stimulating insulin secretion from pancreas [74]. Further, research needs to be carried out to standardize the specific mechanism of action of each active constituent.

**CONCLUSION:** The evergreen plant *Actinodaphne hookeri* Meissn. was known for its anti-diabetic

activity since early times. The results obtained in the present study also revealed that the plant has *in-vitro* and *in-vivo* anti-diabetic activity.

This supports its ethnobotanical claim and use. *In vitro* and *in-vivo* anti-diabetic activity of pet ether extract, chloroform extract, methanol extract, aqueous extract and total extracts of leaves of AH were evaluated. It revealed that chloroform extract, pet ether extract, and total extract had potent hypoglycemic and hypolipidemic activity. The presence of sterols, triterpenes, alkaloids, flavonoids, and tannins were responsible for the hypoglycemic effect. The activity may be due to individual or combined effects of the active constituents. The exact mechanism of action is not known, but it may be due to multiple mechanisms.

Future, prospects for isolation of its phytoconstituents might be promising for the development of phytomedicines for DM and associated complications.

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**CONFLICT OF INTEREST:** The authors have declared that there are no conflicts of interest.

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