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Investigating of Anti-Diabetic Potential of Secondary Metabolites from *Abies Webbiana*

Nasiruddin Ahmad Farooqui[1], Km. Banti[2*], Praveen Kumar[3], Shamim Ahmad[4]

[1,2*,3,4]Translam Institute of Pharmaceutical Education and Research, P.O. Rajpura, Mawana Rd, Meerut, Uttar Pradesh 250001, Email Id: bantigurjar15081998@gmail.com

***Corresponding Author:** Km. Banti
*Email Id: bantigurjar15081998@gmail.com Mob;- +91-9149114415

ABSTRACT
Abies Webbiana, commonly known as the Himalayan silver fir, is a coniferous tree native to the Himalayan region and has been traditionally used in folk medicine. This study aimed to isolate and characterize secondary metabolites from ***Abies Webbiana*** and evaluate their pharmacological activities.
The secondary metabolites were extracted using various solvent systems and then isolated using chromatographic techniques. The isolated compounds were characterized using spectroscopic methods such as nuclear magnetic resonance (NMR) and mass spectrometry (MS).
Our results revealed the successful isolation and characterization of several secondary metabolites from ***Abies Webbiana,*** including terpenoids, flavonoids, and phenolic compounds. These compounds exhibited promising pharmacological activities, including significant antioxidant and anti-inflammatory effects.
This study highlights the pharmaceutical potential of ***Abies Webbiana*** as a source of bioactive compounds with diverse pharmacological activities. Further research is warranted to elucidate the mechanisms of action and therapeutic potential of these isolated secondary metabolites for the development of novel pharmacotherapeutic agents.

Key Words: - Abies *Webbiana,* Diabetic, and NMR etc.

Introduction

A broad spectrum of metabolic diseases signified by elevated blood glucose concentration due to inadequate insulin production or usage are collectively referred to as diabetes mellitus. Chronic hyperglycemia can cause problems with the kidneys, nerves, eyes, and small blood vessels. Diabetes is also frequently linked to other common disorders like heart attacks and strokes. From a social standpoint, diabetes is viewed as a major financial burden because of the high cost of treatment and the early morbidity and death that are linked to it. From the perspective of the individual patient, diabetes is a lifelong condition requiring frequent medication, ongoing attention to nutrition and lifestyle, and regular blood glucose monitoring. It can also lead to different degrees of emotional suffering, such as depression and anxiety, and require several medical visits [1]. According to the WHO, diabetes is a metabolic illness marked by persistently high blood glucose levels. Over time, diabetes can cause damage to the kidneys, heart, and nerves [2]. The normal range for a healthy person's blood glucose level (fasting) is 70–100 mg/dl. If the glucose levels are found to be between 100 and 125 mg/dl, the person is considered prediabetic and needs to modify their lifestyle to prevent or delay the onset of diabetes. If the blood glucose concentration is 126 mg/dl or higher on two different occasions, the person is diagnosed with diabetes [3].

Types of diabetes mellitus

Diabetes mellitus can be classified into four distinct types:

- **Type 1**
- **Type 2**
- **Gestational**
- **others**

Type 1 When beta cells of pancreas are normally destroyed. Due to this degradation, there is a noticeable insulin shortage, and while the rate of advancement may vary, plasma C-peptide levels are typically undetectable.
Type 2 Conversely, T2DM is a complex disorder that is charecterized by differing degrees of insulin resistance in addition to beta cell dysfunction. Insulin resistance is a typical characteristic of various metabolic disorders that may coexist with type 2 diabetes, such as obesity, polycystic ovarian syndrome, and hypertension.

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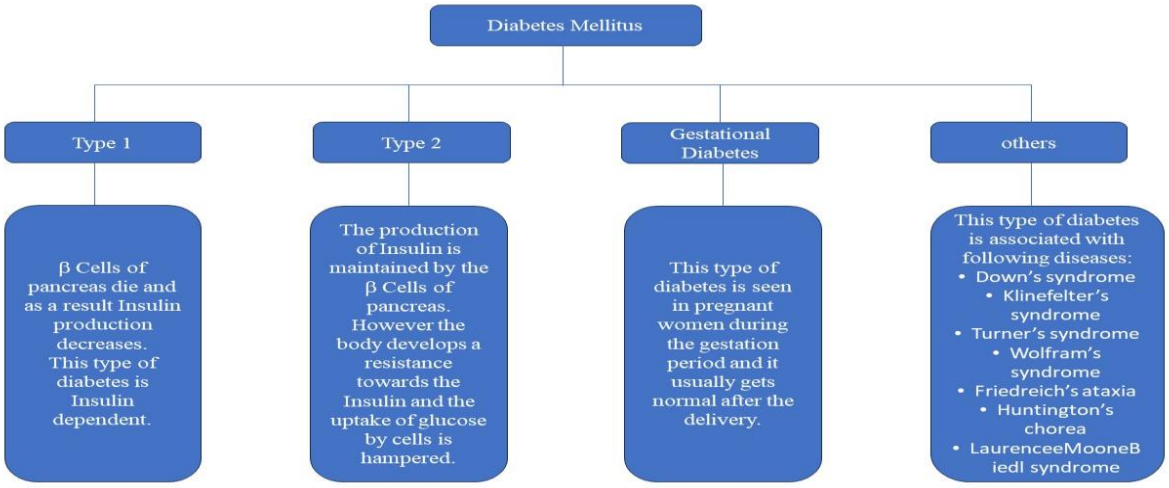
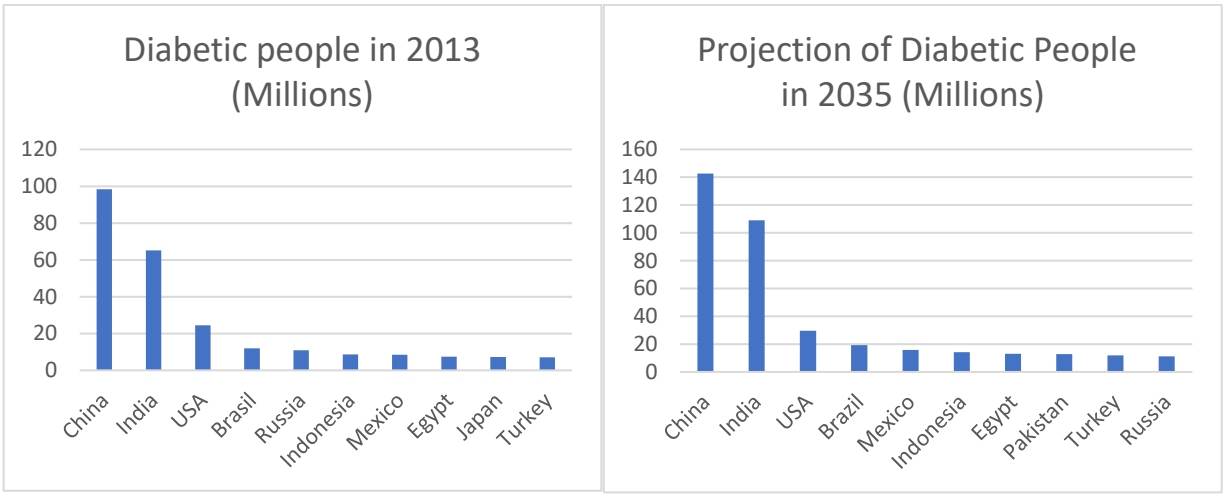


Figure 1: Types of diabetes and their description.

In circumstances where pre-existing diabetes is unlikely, sensitivity to carbohydrates initially identified during pregnancy is referred to as gestational diabetes. Pregnancy-related medical complications are common and are associated with a higher chance of unfavorable outcomes. Even though the illness usually goes away after childbirth, women who are affected should be evaluated for persistent diabetes after giving birth and should be aware of their increased risk of developing type 2 diabetes in the future. Others with the fourth kind of diabetes are linked to different genetic disorders, including Down syndrome, Turner syndrome, and Laurence-Moon-Biedl syndrome; however, it is unclear what causes diabetes in these people in the first place [1].



(A) (B)

Figure 2: (A) Number of individuals suffering from diabetes in 2013 by country; (B) Number of individuals expected to be diabetic by 2035 by country.

Mechanism of Insulin action and its Resistance.

The insulin receptor comprises an extracellular region responsible for binding ligands and an intracellular domain housing the tyrosine kinase. Upon insulin binding to the extracellular segment, the kinase activity of the receptor is triggered, leading to the phosphorylation of specific tyrosine residues within the receptor. This autophosphorylation event facilitates the recruitment and phosphorylation of various scaffolding of proteins like insulin receptor substrates to the intracellular sites of the receptor [5-9].

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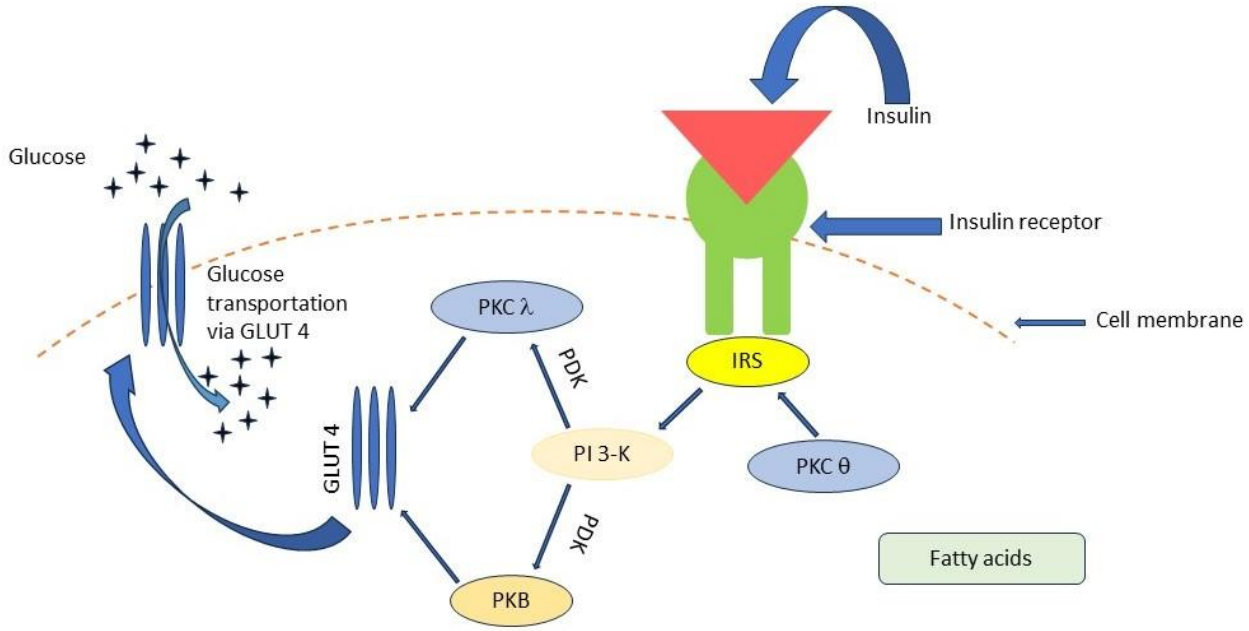

Figure 3: Insulin receptor activation by insulin is the mechanism of insulin absorption via GLUT 4.

Furthermore, evidence links impaired IRS function in skeletal muscle to lipotoxicity and adipocyte physiology. For instance, serine phosphorylation of IRS proteins can be induced by high blood levels of free fatty acids and TNFα, which can obstruct the transmission of insulin signals. Furthermore, prolonged insulin exposure—which is commonly seen in diabetes patients with hyperinsulinemia—may trigger the IRS proteins to break down in a regulated way. IRS proteins possess several contact domains that aid in attracting various signaling molecules, such as phosphoinositide-3-kinase (PI 3-kinase), even though they lack intrinsic enzymatic activity [10-14].

Protein and lipid phosphatases

Metabolic balance requires the cessation of insulin signals. An inhibitory subset of phosphatases inhibits the insulin receptor's signaling cascade. Protein tyrosine phosphatase 1B, or PTP1B, is one of the important phosphatases in this situation. Mice deficient in PTP1B gene, for instance, exhibit enhanced insulin response and do not develop into insulin resistance when fed a high-fat diet. Furthermore, by blocking PTP1B activity, systemic injection of PTP1B-specific antisense oligonucleotides enhanced insulin response and glucose regulation in diabetic rats [23][24][25].

Diabetes mellitus results from resistance against insulin which impairs the intake of glucose by the cells throughout the body. The whole mechanism of glucose uptake is extremely complex and is mediated by a variety of proteins and any malfunction in this machinery can result in development of T2DM. Factors such as obesity and lack of physical activity can lead to increase in free fatty acids in the body which can also impair the uptake of glucose by disrupting this system.

Management of T2DM

The treatment of T2DM starts with change in lifestyle and includes administration of anti-diabetic drugs for the management of diabetes. Diet and exercise are the primary factors influencing energy balance, forming the foundation of diabetes management. Sufficient rest is also crucial for energy levels and overall well-being, with a recommended 7 hours of sleep per night [26]. Research shows that 6 to 9 hours of nightly sleep is associated with reduced cardiometabolic risks[27], while sleep deprivation worsens insulin resistance, hypertension, hyperglycemia, and dyslipidemia [28]. Additionally, patients suspected of having obstructive sleep apnea should undergo screening and be referred to a sleep specialist for evaluation and treatment [26]. While the range of pharmacological options continues to expand, providing more therapeutic possibilities, lifestyle interventions remain essential in managing these patients and achieving therapeutic goals [29]. Intake of macronutrients is crucial for the management of diabetes, it is suggested the individuals with diabetes should avoid carbohydrates as much as possible and limit the overall+ intake of mono and disaccharides, while maintaining the intake of protein between 28% to 40% of diet and also limiting the intake of lipids as they can also contribute to the resistance of insulin [30].

Apart from lifestyle changes hypoglycemic drugs are also essential for the effective management of T2DM. There are various kinds of oral hypoglycemics available for the management of T2DM as depicted in the table 1[31]. Apart from modern drugs herbal natural compounds have also been explored and exhibited antidiabetic activity. Herbal products have a diverse range of phytochemicals present in them which enable them to show vast number of activities by acting upon different and multiple targets simultaneously. Some of the commonly used herbs possess antidiabetic activity.

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Method and materials
All the materials utilized were of laboratory grade and standardized methodology was used for the entirety of the study.
Result and Discussion
Physico-chemical result
Foreign matter
Foreign matter content in the *Abies webbiana* is depicted in the table 5.2.

Table 1.2. Foreign matter of selected plants

S.no	*Abies Webbiana*
1	1.5±0.64

Ash value determination
The ash value outcome are depicted in the table 5.3 and figure 5.1.

Table 5.3. Table depicting various ash values.

Name of the plant	Total ash value	Water soluble ash value	Acid insoluble ash value	Sulphated ash value
Abies Webbiana	0.59 ± 0.28	1.6 ± 0.25	0.47 ± 0.13	0.8 ± 0.38

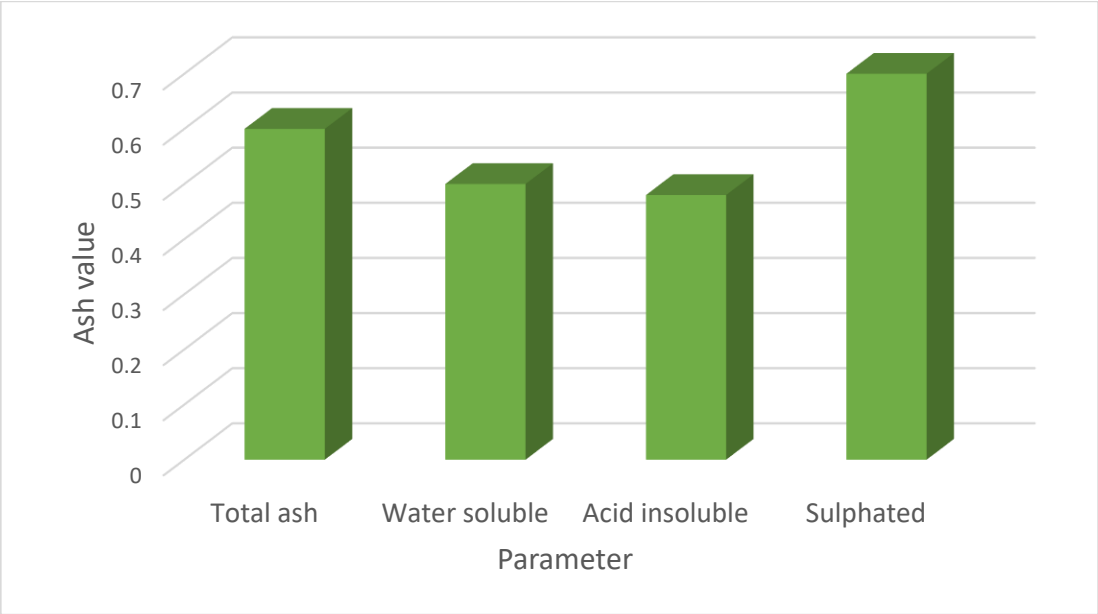

Figure 5.1. Various ash values of *Abies webbiana*

Extractive value

Table 5.4. Extractive value

Plants	*Abies Webbiana*
Alcoholic extract	18.2±0.54
Aqueous extract	16±0.35

Loss on drying (LOD)

Table 5.5. LOD (Loss on drying)

Plants	*Abies Webbiana*
LOD	1.48±0.45

Extraction
Dried leaves powder of were extracted through maceration and the yield is reported in the table 5.6.

Table 5.6. Percentage yield

Plants	*Abies Webbiana*
Percentage yield(%w/w)	2.2

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Fluorescence analysis

Another useful technique for figuring out what ingredients are in herbal medications is the fluorescence analysis. The drug powder was subjected to a variety of chemical reagent applications before the powder was used for research. Both visible and ultraviolet light were used to help with the investigation.

Table 5.7. Fluorescence evaluation of *Abies Webbiana*

Treatment	*Abies Webbiana*	
	White light	**UV light**
Dried leaf powder	Creamy	Bright white colour
Crude drug + H_2O	Dark green colour	Dark green colour
Crude drug + Methyl alcohol	Brown colour	Light green colour
Crude drug + HCL	Creamy Colour	White colour
Crude drug + NaOH Aqueous	Light brown colour	Green colour
Crude drug + HNO_3 80%	Soil colour	Black colour
Crude drug + NaOH alcoholic	Light brown colour	Green colour
Leaves Powder +50% H_2SO_4	Dark brown colour	Black colour

Phytochemical Screening

Qualitative chemical tests

Bioactive ingredients including terpenoids, flavonoids, alkaloids, tannins, phenols and glycosides have been identified to be present in the leaves extract using phytochemical screening.

Table 5.8. Phytochemical evaluation of *Abies Webbiana*

Phytochemical Test	Petroleum ether	Ethyl acetate	Methyl alcohol
Test for carbohydrates			
Molish test	Negative	Positive	Positive
Fehling's test	Negative	Positive	Positive
Benedit's test	Negative	Negative	Positive
Test for alkaloids			
Mayer's test	Negative	Positive	Positive
Hager's test	Negative	Positive	Positive
Wagner's test	Negative	Positive	Positive
Terpenoids & Steroids			
Salwoski	Negative	Positive	Positive
Libberman Burchard	Negative	Negative	Negative
Flavonoids			
Lead acetate	Negative	Positive	Positive
Alkaline reagent test	Negative	Positive	Positive
Tannin & Phenolics			
Ferric chloride	Negative	Positive	Positive
Lead acetate	Negative	Positive	Positive
Gelatin	Negative	Positive	Positive
Saponins			
Froth	Negative	Negative	Negative
Protein & amino acids			
Biurets	Negative	Negative	Negative
Ninhydrin	Negative	Negative	Negative
Glycosides			
Borntrager	Negative	Positive	Positive
Legal test	Negative	Positive	Positive
Killer killani	Negative	Positive	Positive
Fats			
Spot test	Positive	Negative	Negative

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Quantitative Phytochemical Screening:
Estimation of tannins
Table 5.9. Tannins of the selected plants

Name of the plant	Tannins
Abies webbiana	1.5-2.0%

Estimation of total phenolic content
Table 5.10. Calibration curve of Propanoic acid

Serial No.	Concentration (µg/mL)	Absorbance
1	0	0
2	0.5	0.07
3	2	0.137
4	4	0.21
5	6	0.282
6	8	0.35
7	10	0.45
8	12	0.55
9	14	0.62
10	16	0.72
11	18	0.825
12	20	0.999

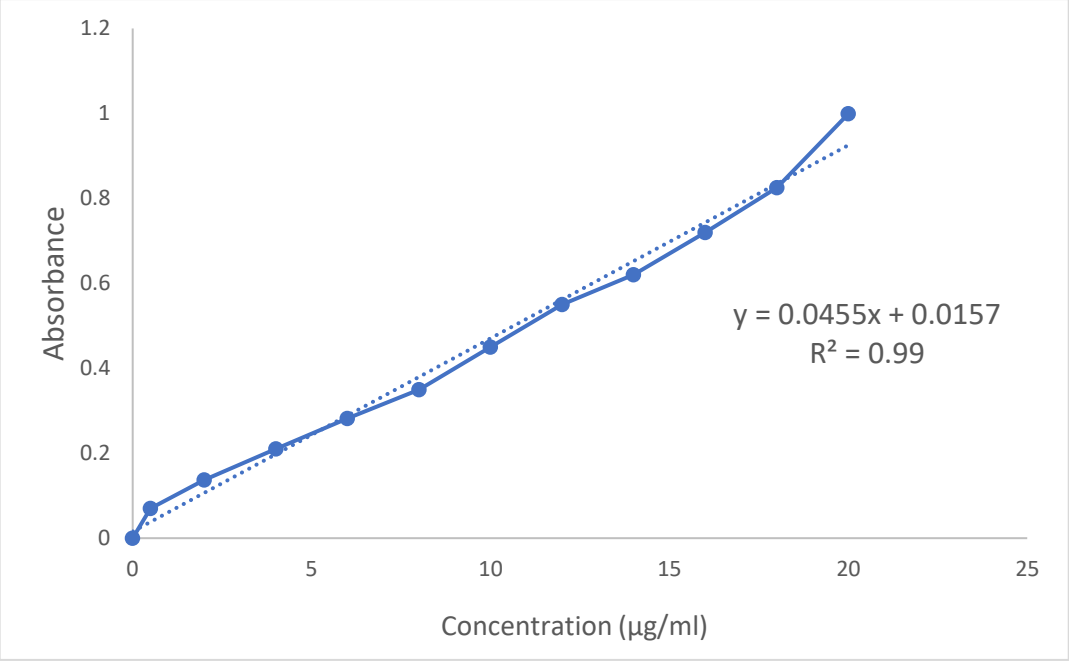

Figure 5.2 Calibration curve of Propanoic acid

Table 5.11. Results of Total phenolic contents of selected plants

Solvents	*Abies Webbiana*
Petroleum ether	35.06±5.41
Chloroform	71.66±5.04
Ethyl acetate	250.01±6.59
Butanol	98.58±2.79
Water	38.59±2.79
Methanol Extracts	26.67±2.78

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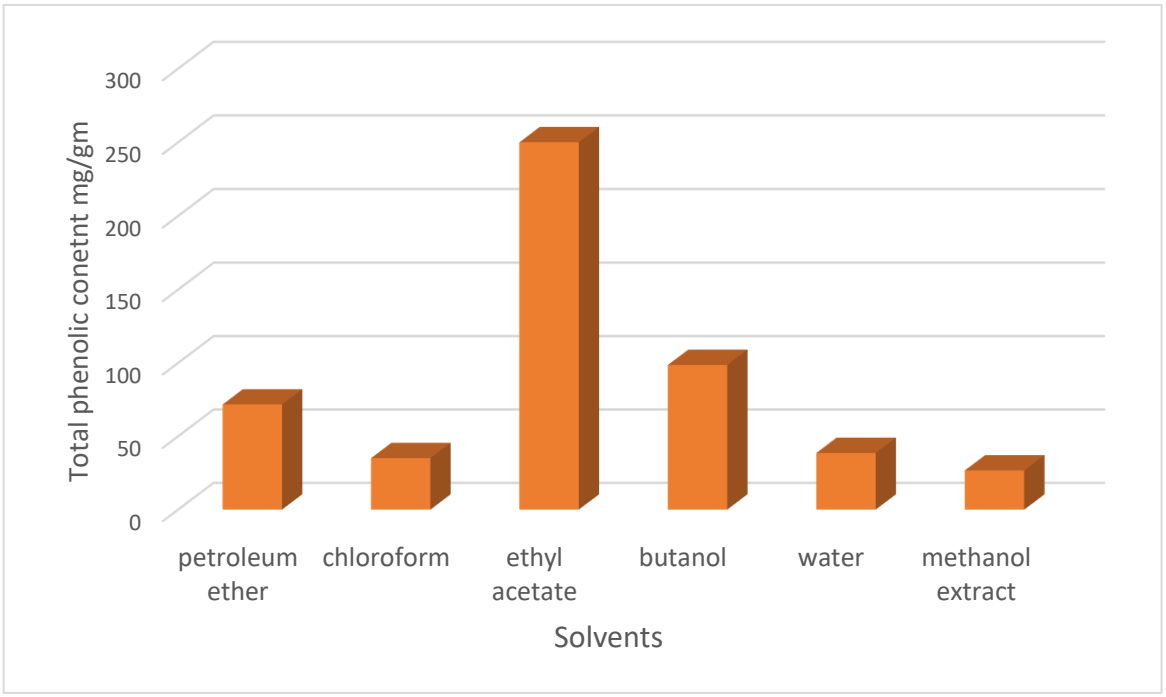

Figure 5.3 *A. webbiana* Phenolic content

Total flavonoid estimation

Table 5.12. Calibration curve of Methoxy quercetin

Concentration (µg/ml)	Absorbance
zero	0
0.5	0.11
2	0.21
4	0.298
6	0.45
8	0.55
10	0.711
12	0.825
14	0.946
16	1.064
18	1.185
20	1.29

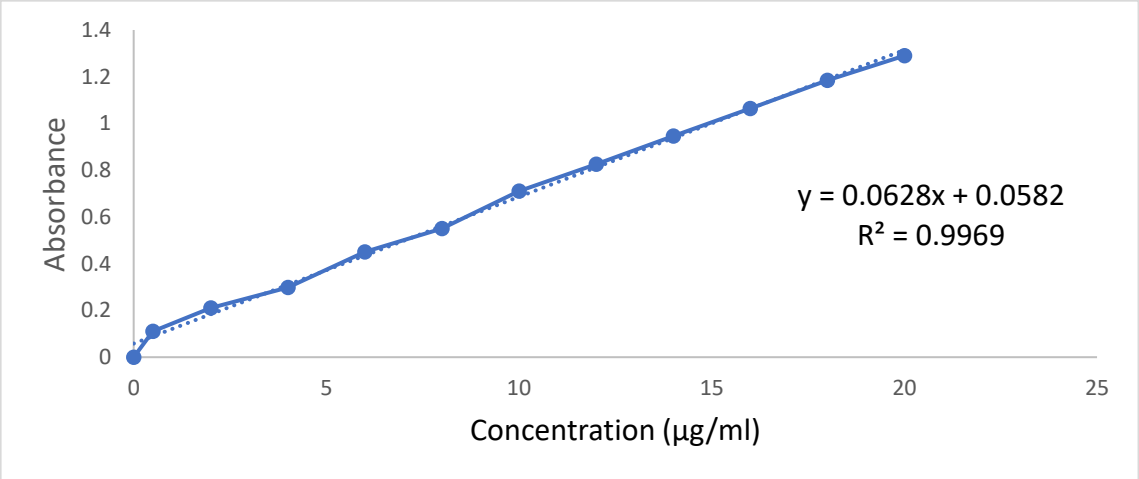

Figure 15.4 Calibration curve of Methoxy quercetin

Table 5.13. Results of Total flavonoid contents of selected plant

Fractions	***Abies webbiana***
Petroleum ether	91.66±5.04
Chloroform	55.06±5.41
Ethyl acetate	310.01±6.59
Butanol	99.58±2.79
Water	40.59±2.79
Methanol extract	35.67±2.78

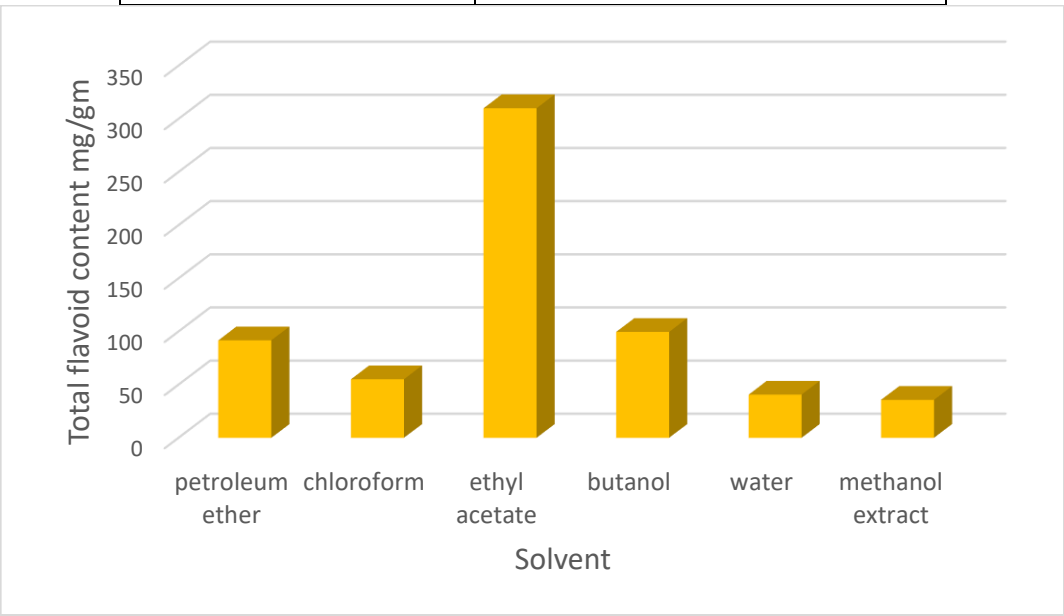

Figure 5.5. *Abies webbiana* total flavonoid content

Standardization of extracts
HPLC

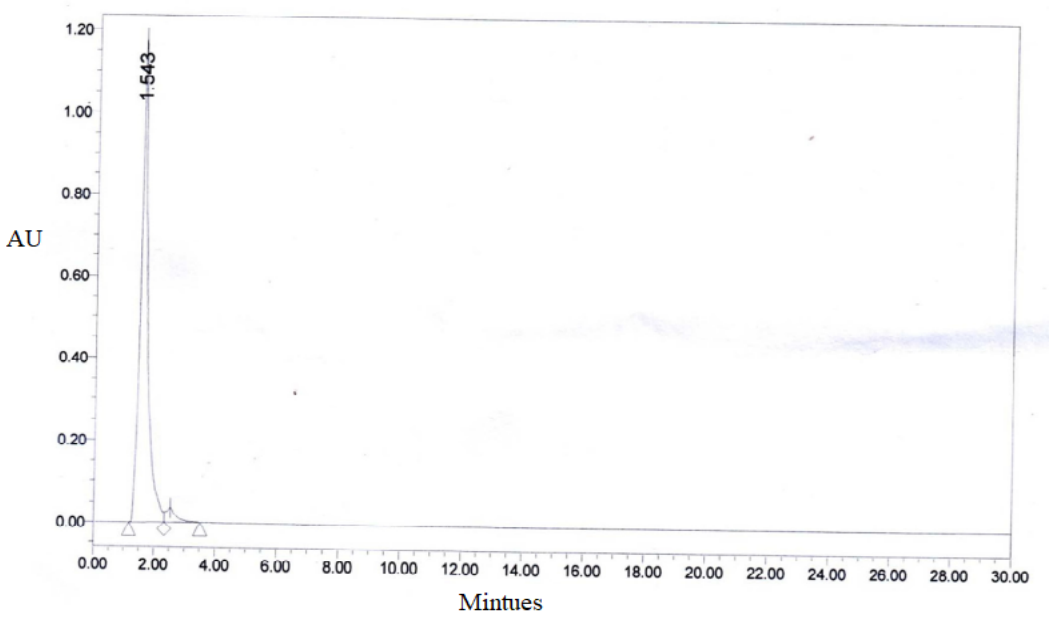

Figure 2.Chromatogram of extract of *Abies webbiana*

Plant extract was subjected to standardization. The extracts' HPLC chromatogram showed a peak at Rt 1.543 with 97.12% of the area occupied.

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Standardization of selected plant extract by HPTLC
Abies Webbiana

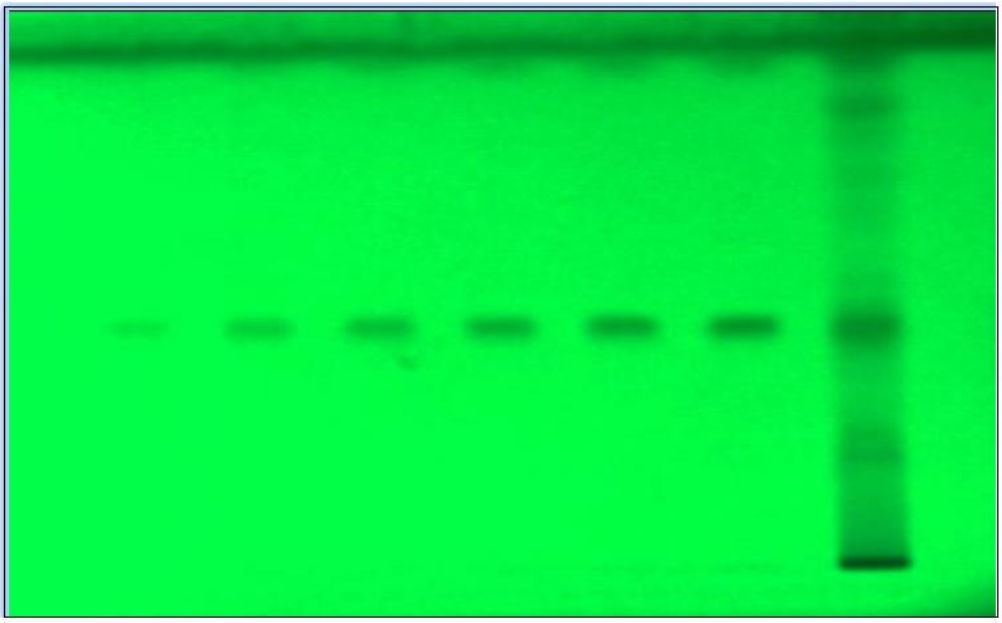

Figure 5.7. HPTLC plate depicting *Abies Webbiana* (200 - 700ng/spot) R_f value was calculated 0.66 ± 0.03

Anti-oxidant assay
DPPH assay

The aqueous extracts of Abies Webbiana have an IC_{50} value of 78.20 µg/ml. Proton radical scavenging is the primary technique for measuring antioxidant activity. The amount of DPPH in the antioxidant is measured using this assay. Every extract in the current analysis exhibits a higher inhibitory percentage.

Table 2. DPPH assay activity of aqueous extracts of *Abies Webbiana*

Concentration (µg/ml)	% DPPH scavenging	IC_{50}
10	20.7±0.84	78.20
20	24.1±0.68	
30	28.6±0.61	
40	32.8±0.64	
50	37.1±0.31	

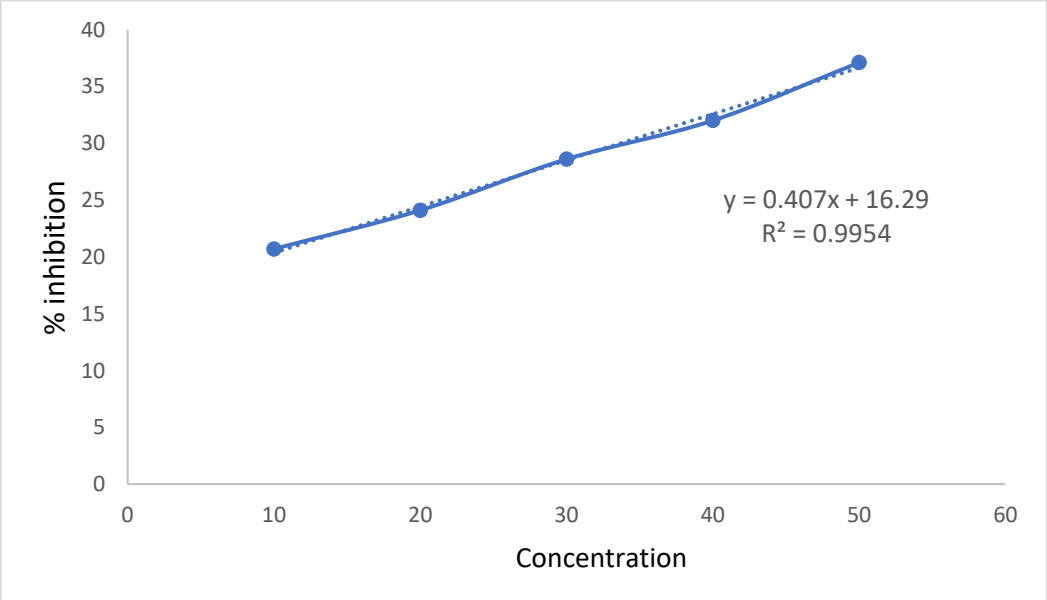

Figure 3.8. *Abies Webbiana* aqueous extract's DPPH test activity

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Hydrogen peroxide radical scavenging activity

The results were expressed as the IC50 value and were obtained using aqueous extracts of Abies Webbiana. Every extract and reference component, such as methyl quercetin, has its IC50 determined. For Abies Webbiana, the IC50 value was 55.39 µg/ml. Hydraulic extracts' H2O2 radical activity. As compared to other extracts, Abies Webbiana exhibits strong antioxidant activity.

Table 5.15. Various H2O2 radical concentrations in Abies Webbiana aqueous extracts

Concentration (µg/ml)	% H_2O_2 scavenging	IC_{50}
10	28.4±0.42	55.39
20	35±0.81	
30	36.9±0.37	
40	42.3±0.52	
50	48.1±0.81	

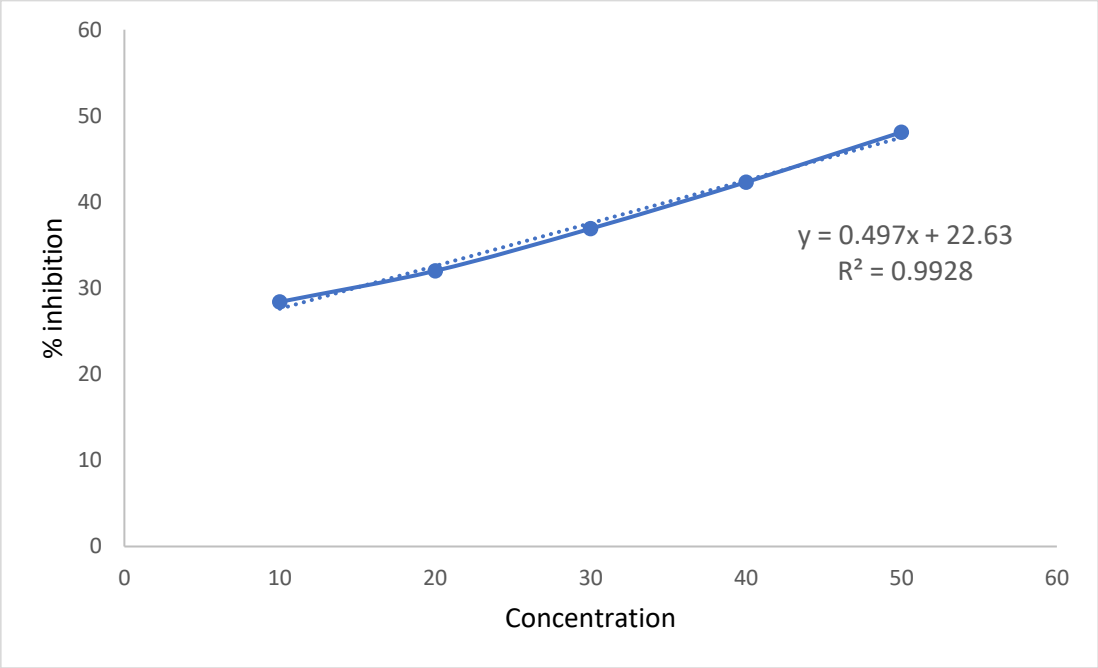

Figure 5.9. Various H2O2 radical concentrations in abies webbiana aqueous extracts

Nitric Oxide Scavenging

Aqueous extracts of Abies Webbiana were used, and the results were expressed as an IC50 value. *Abies Webbiana's* nitric oxide IC_{50} value was 46.6 microgram/ml.

Table 5.36. Different concentration of aqueous extracts of *Abies Webbiana*

Concentration (µg/ml)	% Nitric oxide scavenging	IC_{50}
10	27.3±0.47	46.66
20	32±0.53	
30	38.7±0.29	
40	46.3±0.38	
50	50.8±0.47	

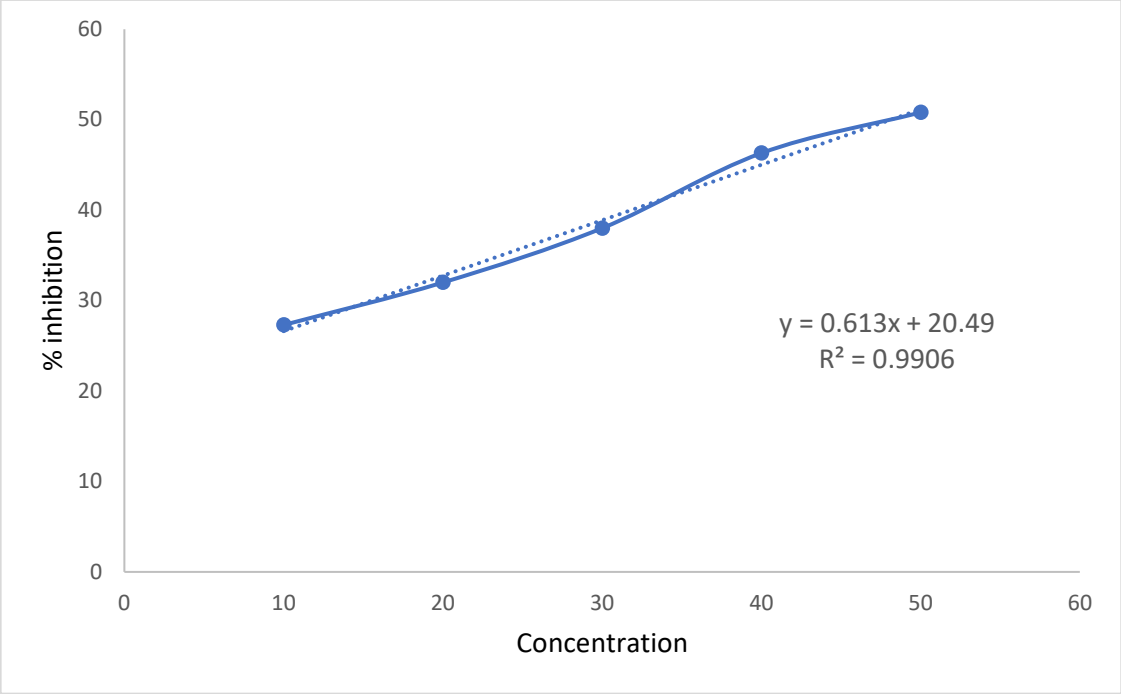

Figure 5.10. Scavenging effect of aqueous extracts of *Abies Webbiana* on nitric oxide assay

***In vivo* study of Anti-diabetic activity of the extract (s)**

Sample preparation (SE) tolerance effects on glucose

There was a spike in glucose levels in the control group. The group treated with glibenclamide has a lower plasma glucose level. The prepared dose of the sample demonstrated effective glucose tolerance. When compared to the conventional dose, the produced dose exhibits the best glucose tolerance characteristics.

Table 5.17. Oral glucose tolerance effects of prepared dose

Group	Plasma glucose concentration (mg/dl)		
	0 min	**30 min**	**90 min**
Normal Control	95.1±5.2	94.7±6.3	96.4±7.4
Glucose control	80.4±6.3	203.1±9.2	182.5±5.3
Glucose + Glibenclamide (0.5 mg/kg)	75.7±3.7	107.4±5.3	150.2±8.1
SE (100mg/kg)	50.63±0.31	52.6.63±0.31	54.6±0.31
SE (200mg/kg)	48.8±0.19	45.8±0.25	44.85±0.58
SE (300 mg/kg body weight)	15.68±6.8	15.25±6.8	16.65±7.3
SE (400 mg/kg body weight)	12.65±7.2	13.85±5.4	12.65.7±6.8

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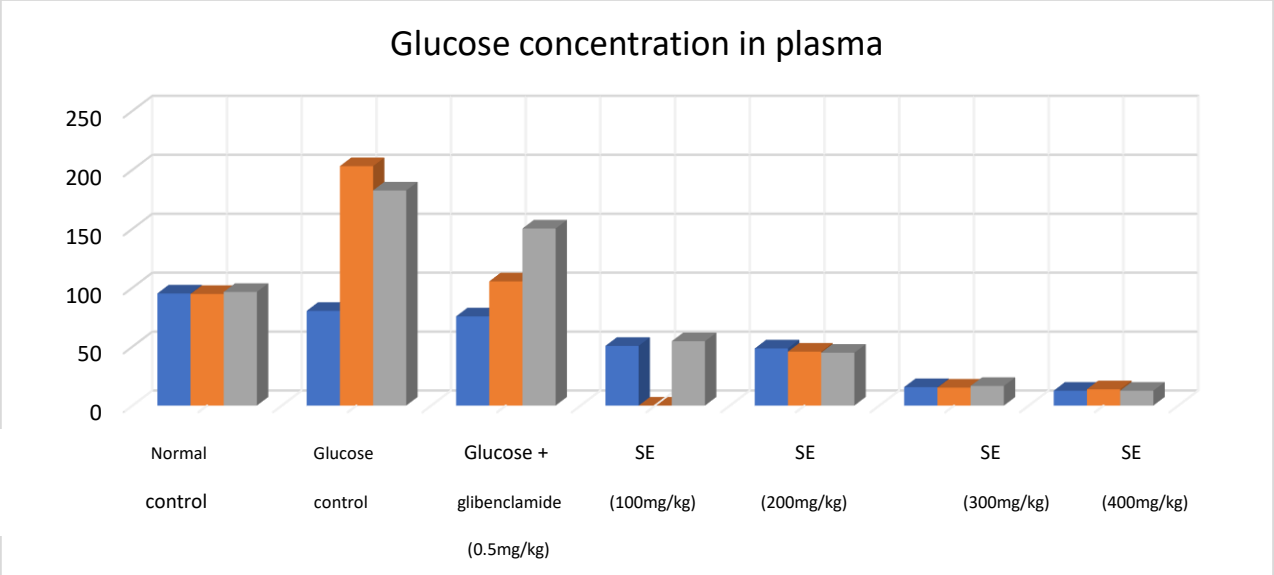

Figure 4. Plasma glucose concentration

Effects on NIDDM

The formation of diabetes in experimental rats is proven as a result of the increased fasting. From day 0 to day 28, there was a rise in the glucose levels in the streptozotocin control groups. Glibenclamide treatment caused a drop in serum glucose levels in the treated rats. The amount of glucose decreases in the mice treated with SE.

Table 5.18. Effect on diabetes mellitus

Group	Level of blood glucose (Fasting) (mg/dl)			
	0^{th} day	**7^{th} day of the study**	**14^{th} day of the study**	**28^{th} day of the study**
Control group (Normal)	82.6±7.5	76.3±5.3	83.7±7.1	87.8±4.8
Diabetic control (Streptozotocin)	191.2±6.8	253.1±9.2	301.6±5.4	354.5±7.1
Standard Control administered with Glibenclamide (0.50 mg/kg)	173.7±9.9	114.5±9.3	95.7±5.4	78.4±8.1
SE (100 mg/kg body weight)	95.3±6.2	91.8±7.1	90.5±6.2	89.4±8.3
SE (200 mg/kg body weight)	65.2±7.4	62.9±8.4	61.3±9.7	60.1±6.5

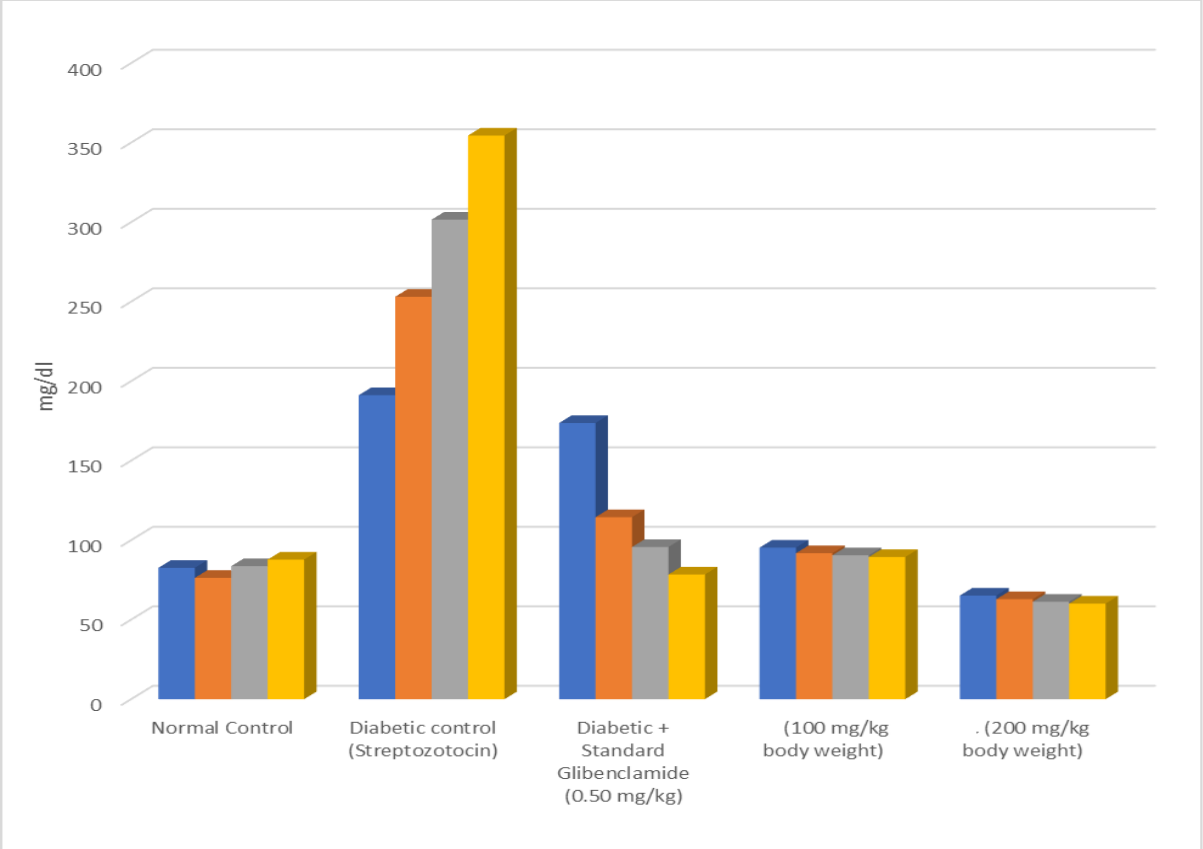

Figure 5.12. Effect on diabetes mellitus

Hyperlipidaemic activity of SE

Those animals which were treated with SE shows decrease in TGL, LDL, total cholesterol but there is a slightly increase in HDL. This observation is done on 28th day of experiment.

Table 5.19. Anti-hyperlipidaemic activity

Group	**Lipid Profile (mg/dl)**			
	Levels of triglycerides	**Total cholesterol**	**High density lipoprotein**	**Low density lipoprotein**
Control (Normal)	81.3±5.3	92.5±4.7	76.2±7.6	58.8±6.2
Negative Control or diabetic control	227.8±6.1	205.1±5.3	19.6±3.8	219.8±4.7
Standard control administered with Glibenclamide (0.50 mg/kg)	89.8±6.1	88.1±6.3	78.4±7.7	66.1±5.2
SE(100 mg/kg)	85.2±5.9	84.8±4.8	91.2±6.2	56.2±5.1
SE (200 mg/kg)	80.3±7.2	86.2±6.4	90.8±4.1	53.1±3.7

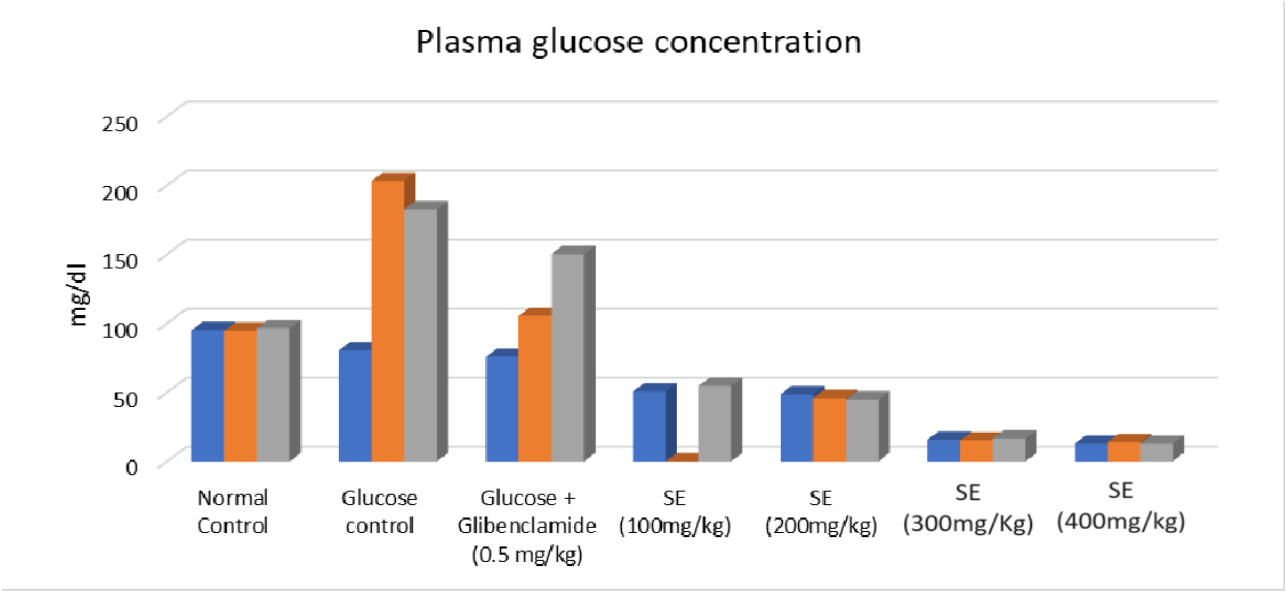

Figure 5. Anti-hyperlipidaemic activity of SE

Effect on body weight

Table 5.20. Effect on body weight

Group	Body weight deviation (gm)		
	Prior to induction of diabetes	Post induction of diabetes	Post treatment
Control (Normal)	187.2 ± 2.7	195.4 ± 4.6	181.7 ± 1.8
Negative Control or diabetic control	181.1 ± 4.1	124.6 ± 2.8	101.4 ± 3.5
Standard control administered with Glibenclamide (0.50 mg/kg)	191.3 ± 5.6	143.7 ± 8.2	190.4 ± 2.4
SE(100 mg/kg)	179.5 ± 2.5	185.2 ± 3.2	184.7 ± 2.7
SE (200 mg/kg)	186.3 ± 3.9	195.2 ± 1.8	189.1 ± 4.5

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