How to Cite:
Dixit, P. K., Nagarajan, K., & Kumar, S. (2022). Antidiabetic activity of Ayurvedic polyherbal formulations Avipattikara Churna and Triphla Churna in streptozotocin and nicotinamide induced diabetic wistar rats. *International Journal of Health Sciences*, *6*(S6), 4582–4606. https://doi.org/10.53730/ijhs.v6nS6.10686

Antidiabetic activity of Ayurvedic polyherbal formulations Avipattikara Churna and Triphla Churna in streptozotocin and nicotinamide induced diabetic wistar rats

Praveen K. Dixit
Department of Pharmacology, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad-201206, Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, U.P
*Corresponding author email: praveendixit87@gmail.com

K. Nagarajan
Department of Pharmaceutical Chemistry, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad-201206, Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, U.P

Sokindra Kumar
Department of Pharmacology, Kharvel Subharti College of Pharmacy, Meerut, U.P-250005

***Abstract*---**Objective: The aim of the present study is to formulate a polyherbal formulation whose formula is given in AFI and evaluate its antidiabetic potential in animals. Method: The polyherbal formulation was prepared using the methanol extracts and aqueous extracts of the given parts of herbs in AFI. The quality of the finished product was evaluated as per the World Health Organization's guidelines for the quality control of herbal materials. The quality testing parameters of the polyherbal formulation were within the limits. Results: The acute toxicity studies of the polyherbal formulation did not show any toxic symptoms in doses up to 2000 mg/kg over 14 days. The extracts of both the formulations were evaluated for In-vitro and In-vivo anti-diabetic study. The oral anti-diabetic activity of the polyherbal formulation (200 and 400 mg/kg, p.o) was screened against intraperitoneal (i.p.) STZ (35 mg/kg) 15 min after i.p. administration of nicotinamide (200 mg/kg) continuously for 2 days, to induced diabetes mellitus in Wistar rats. The investigational drug was administered for 28 consecutive days, and the effect of the polyherbal formulation on blood glucose levels was studied at regular intervals. At the end of the study, the blood samples were collected from all the

animals for biochemical estimation, and the animals were sacrificed, and the kidney, liver and pancreatic tissues were collected for histopathologic analysis. Conclusion: Polyherbal formulations showed significant antidiabetic activity at 200 and 400 mg/kg, respectively, and this effect was comparable with that of Glibenclamide. The antidiabetic activity of polyherbal formulation was supported by In-vitro assessment and histopathological analysis.

***Keywords*---**streptozotocin, glibenclamide, nicotinamide, avipattikara churna, triphla churna.

Introduction

Since ancient time plants are very useful to human fraternity. Most of them are frequently and exclusively used for medicinal purposes. According to the World Health Organization (WHO), "a medicinal plant is a plant which, in one or more of its parts, contains substances that can be used for therapeutic purposes, or which are precursors for many pharmaceutical products." Such plants are in huge demand by pharmaceutical companies for their active ingredients. [1,2] *Avipattikara Churna* and *Triphala Churna* are the ayurvedic formulations which are used to treat Prameh. There are twenty types of Pramehas (Kaphaja-10 Pittaja-6, and Vataja-4) have been mentioned in the Ayurveda. 'Prameha' and 'Diabetes' are synonymous. Diabetes is one of the 'Prameha' which consists of two words, 'Pra' meaning abundant, and 'Meha' meaning 'passing of large quantity of Urine. Incidentally, the term diabetes has been derived from the Greek term 'Diabainein' to mean 'to cross through a siphon' meaning the continuous free flow of water and applied to mean the elimination of large quantity of Urine.[3] Thus the terms 'Prameha' and 'Diabetes' carry similar meaning.

Diabetes mellitus (DM) belongs to the class of metabolic diseases having the primary symptom associated with this disease is high sugar levels in the blood for an extended period and polyurea is one of the prominent symptoms which imbalances fluid, hence reduces plasma level in the body. It can be categorized to the world's major diseases considering that affects the high population on the earth and presents two main types I and II. The prevalence of diabetes for all age-groups world-wide was estimated to be 2.8% in 2000 and 4.4% in 2030. Diabetes complications include possible blindness, amputation of a lower limb, renal failure, and cardiac arrest or stroke. Till now, various kinds of synthetic and herbal formulations were made, and many are more frequently used to achieve desirable treatment. Patients prefer oral anti-diabetic medications since they are more comfortable to be administered, and for this reason, researchers focus their studies in this direction**.** [4] This research work aimed to explore the possibility of anti-diabetic treatment from Polyherbal sources well mentioned in Ayurvedic Formulary of India. *Avipattikara churna* is a herbal remedy used in Ayurveda, the traditional medicine of India. Available in dietary supplement form, it contains several different ayurvedic herbs and is generally used to GIT problems. Several preliminary studies show that some of the herbs found in Avipattikara may aid in the treatment of certain health conditions. For instance, there's some evidence that amla may help keep cholesterol and blood pressure in check and protect

against Prameha (diabetes, while haritaki may reduce oxidative stress and help treat metabolic syndrome. *Triphala churna* is a well-known traditional ayurvedic formulation which is most commonly used to tone up and support the normal functioning of the bowel. Many traditional compounds containing Triphala as an ingredient, is found to be useful in the treatment of several kinds of ailments and diabetes is among one of them. Triphala has been described elaborately in Ayurveda for the treatment of one of the Prameha (diabetes mellitus). Triphala have inhibitory potential of pancreatic glycolytic enzymes, namely alpha-amylase and alpha-glucosidase, which break down larger polysaccharides into glucose molecules that enter the blood stream.[5]

Material and Methods

Collection of the plant

All the constituent herbs used in the formulation of *Avipattikara Churna* and *Triphala Churna* has been procured from Arya Vastu Bhandar, Dehradun and Herbal Market, Khari Bawli, New Delhi. The herbs were authenticated by Dr. Sunita Garg, Emeritus Scientist of Raw Materials Herbarium and Museum Department (RHMD) of National Institute of Science Communication and Information Resources (NISCAIR),NewDelhi with reference number: NISCAIR/RHMD/Consult/2019/3560-61-1 to 12.

Animals

Adult Wistar rats (190 ± 10 gm b.w) of either sex were procured from Animal House Facility of All India Institute of Medical Sciences (AIIMS) New Delhi .The animals were housed in Animal House Facility of KIET School of Pharmacy, KIET Group of Institutions,Delhi-NCR,Ghaziabad-201206 and maintained under standard conditions of humidity, temperature (25±2 ◦C) and light (12 h light/dark). They were fed with water ad libitum and standard rat pellet diet. The study was approved by the Institute Animal Ethics Committee of KIET School of Pharmacy with Protocol No- IAEC/KSOP/E/20/07 and all the animal experiments were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Ministry of Environment and Forests, Government of India

Physicochemical analysis

The determination of various physicochemical parameters such as foreign matter, extractive values in different solvents, total ash, acid insoluble ash, water-soluble ash, moisture content or loss on drying and pH value of 1% and 10% solutions was carried out by the methods given in the WHO guidelines for standardization of herbal drugs.

Preparation of Polyherbal formulations [6]

Preparation of Avipattikara Churna (F1) [Table-1]

The churna was prepared as per the procedure given in Ayurvedic Formulary of India. All the ingredients viz., *Zingiber officinale, Piper nigrum, Piper longum,*

Terminalia chebula, Terminalia bellirica,Phyllanthus emblica ,Cyperus rotundus, salt (vida lavana), *Embelia ribes, Elettaria cardamomum, Cinnamomum tamala, Syzgium aromaticum, Operculina turpethum*, and *Saccharum officinarum* were powdered separately, passed through 80 # sieve and then mixed together in specified proportions to get uniformly blended churna.

Preparation of Triphala Churna (F2) [Table-2]

The churna was prepared as per the procedure given in Ayurvedic Formulary of India. All the ingredients viz., *Terminalia chebula, Terminalia bellirica, Phyllanthus emblica,* were powdered separately, passed through 80 # sieve and then mixed in specified proportions to get uniformly blended churna.The quality of the polyherbal formulation was tested as per the WHO guidelines for the quality control of herbal materials. As per the guidelines, specific tests such as, ash content, extractable matter, loss on drying and pH were recorded.

Table 1
Composition of Avipattikara Churna

S. No	Ayurveda Name	Hindi Name	Botanical Name	Part Used	Quantity
1.	Sunthi	Adrak Dry	*Zingiber officinale Rosc.*	Rhizome	1 part
2.	Marica	Kali Mirch	*Piper nigrum Linn.*	Fruit	1 part
3.	Pippali	Long Pipper	*Piper longum L.*	Fruit	1 part
4.	Haritaki	Haritaki	*Terminalia chebula Retz.*	Plant (Fr)	1 part
5.	Bibhitaka	Bahera	*Terminalia bellirica (Gaertn.)Roxb.*	Plant (Fr)	1 part
6.	Amalaki	Amla	*Phyllanthus emblica L.*	Plant (Fr)	1 part
7.	Musta	Nut Grass	*Cyperus scariosus R.Br.*	Rhizome	1 part
8.	Vida(Vida Lavana)	Vida Lavana	*Ammonium chloride*	Salt	1 part
9.	Vidanga	Vidanga	*Embelia ribes Burm.f*	Fruit	1 part
10.	Ela(Suksmaila)	Elaichi	*Ellettaria cardmomum (L.)*Maton	Fruit (Seed)	1 part
11.	Patra (Tej patra)	Tej patra	*Cinnamomum tamala* Nees & Eberm.	Leaf	1 part
12.	Lavanga	Clove	*Syzygium aromaticum (L.)Merr*	Flower bud	11 part
13.	Trivrit	Nishoth Kala	*Operculina turpenthum (Linn.)*	Root	44 part
14.	Sarkara	Gud	*Saccharum officinarum*	Gud	66 part

Table 2
Composition Of Triphala Churna

1.	Pathya (Haritaki)	Haritaki	*Terminalia chebula Retz.*	Plant (Fr.)	1 part
2.	Bibhita (Bibhitaka)	Bahera	*Terminalia bellirica (Gaertn.)Roxb.*	Plant(Fr.)	1 part
3.	Dhatri (amalaki)	Amla	*Phyllanthus Emblica L.*	Plant(Fr.)	1 part

Extract Preparation for activity

In present study, plant material was extracted by using cold maceration method. About 2kg of the powder was extracted with two solvents methanol and aqueous, allow standing for 4-5days each. The extract was filtered through whatman no.1 filter paper to remove all unextractable matter. Extract was transferred to beaker and evaporated & excessive moisture was removed and extract was collected in air tight container.

Determination of Moisture Content [7]

Take 2gm of crude drug sample (*Avipattikara Churna* and *Triphala Churna*) into the petri disc and then heat this into hot air oven (105˚C) for 1 hour. Take out petric disc from the oven and allowed to cool down. Calculate the total moisture by using this formula:

% Moisture = (Weight of petri dish + Weight of Sample – Dried weight) X 100 / Weight of Sample

Determination of Extractive Values

5gm of fresh dried powdered drug(*Avipattikara Churna* and *Triphala Churna*) macerate in 100ml of (Methanol, Water, Chloroform, Pet. ether) in a close flask and leave for 24hr. Shake the flask starting 6hr and then leave for 18hr. Filter the whole mixture. 25ml of filtrate evaporate up to dryness at 105^0C. Calculate the total % of obtained extract by using the formula:

(Weight after drying - Weight of petridish) x 100 x 100 / weight of sample x vol. of filtrate

Determination of Ash Values

Take 2gm of crude drug ((*Avipattikara Churna* and *Triphala Churna*) in pre-weigh silica disc and burn this at temperature (not exceeding, more than) 450^oC for 5hr.Calculate the total ash by using the formula:

Ash Value = (Weight after drying - Weight of crucible) x 100 / weight of sample

Acid Insoluble Ash value

Soluble total ash in 25ml of 2M HCL and then filter this by using the ash less filter paper. Wash filter paper till filtrate become neutral. Burn this for 15min at 450^0c and allow to-cool in a desiccator. Calculate the acid insoluble ash by using the formula:

(Weight after drying - Weight of crucible) x 100 / weight of sample

Acute oral toxicity

Acute oral toxicity of the polyherbal formulation was carried out as per the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423. The principle involves a stepwise procedure with the use of a minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance to enable its classification. Healthy Wistar rats (3 animals/dose) of either sex were used for the experiment. Overnight fasted rats were orally fed with the plant extracts and polyherbal formulation in increasing dose levels of 5,50, 300, and 2000 mg/kg body weight, respectively. The animals were observed for their behavioural (alertness, restlessness, irritability, and fearfulness), neurological (spontaneous activity, reactivity, touch response, pain response, and gait), and autonomic (defecation and urination) profiles continuously for 24 h. After a period of 24 h, the animals were observed for 14 days for mortality**.** [8]

In vitro Anti-diabetic activity
In vitro α-Amylase Inhibitory Assay

The assay was carried out following the standard protocol with slight modifications. Starch (2 mg) was suspended in 0.2 mL of 0.5M Tris–HCl buffer (pH 6.9) containing 0.01 M CaCl2 (substrate solution). The tubes containing substrate solution were boiled for 5 min and then preincubated at 37°C for 5 min. Sample was dissolved in DMSO in order to obtain concentrations of 20, 40, 60, 80, and 100 µg/mL. Then, 0.2 mL of sample of particular concentration was added to the tube containing the substrate solution. In addition, 0.1 mL of porcine pancreatic amylase in Tris–HCl buffer (2 units/mL) was added to the tube containing the plant extract and substrate solution. The reaction was carried out at 37°C for 10 min. The reaction was stopped by adding 0.5 mL of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of resulting supernatant was measured at 595 nm using spectrophotometer (Shimadzu UV–VIS spectrophotometer). Acarbose, a known α-amylase inhibitor was used as a standard drug. The experiments were repeated thrice. The α-amylase inhibitory activity was calculated by using following formula. [9]

[1] %Inhibition = (Control – Sample/Control) *100

***In vitro a-glucosidase* inhibitory activity**

α-glucosidase inhibitory activity of extracts was carried out according to the standard method with minor modification. [10] In test tube, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6. 8), 10 µl alpha-glucosidase (1 U/ml), and 20 µl of varying concentrations of extracts 20, 40, 60, 80, 100 µg/ml was pre-incubated at 37°C for 15 min. Then, 20 µl P- NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50 µl Na 2CO3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using UV Vis Spectrophotometer. Acarbose at various concentrations (20–100 µg/ml) was included as a standard. Without test substance was set up in parallel as a control and each experiment was performed

in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula-

[2] %Inhibition = (Control – Sample/Control) *100

Animals and treatment

***In vivo* antidiabetic activity : Experimental induction of diabetes**

Adult male Wistar rats were allowed to acclimatize to the laboratory environment for 12 Days. Six rats in each group were randomly separated and fed on a normal diet. The animals were fasted for 12 h and then received an intra-peritoneal injection of streptozotocin (STZ) (Sigma-Aldrich, USA), dissolved in 0.1 M citric acid/sodium citrate buffer, pH 4.5 at a dose of 60 mg/kg. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h after STZ administration. Rats with fasting blood sugar levels around 160 to 300 mg/dl were selected for the study. [11]

- Normal Control (0.9 % NaCl) for 28 days,
- Diabetic control STZ (35 mg/kg) b.w.i.p in 0.1 M citrate buffer (pH 4.5) in a volume of 0.5 ml/kg b.wt. intraperitoneal (i.p.) 15 min after i.p. administration of nicotinamide (200 mg/kg) continuously for 2 days
- Standard Drug (Glibenclamide 5 mg/kg b.w.p.o for 28 days
- Aqueous Extract Low Dose (AELD-F1) 200 mg/kg b.w.p.o for 28 days
- Aqueous Extract High Dose (AEHD-F1) 400 mg/kg b.w.p.o for 28 days
- Aqueous Extract Low Dose (AELD-F2) 200 mg/kg b.w.p.o for 28 days
- Aqueous Extract High Dose (AEHD-F2) 400 mg/kg b.w.p.o for 28 days
- Methanolic Extract Low Dose (MELD-F1) 200 mg/kg b.w.p.o for 28 days
- Methanolic Extract High Dose (MEHD-F1) 400 mg/kg b.w.p.o for 28 days
- Methanolic Extract Low Dose (MELD-F2)200 mg/kg b.w.p.o for 28 days
- Methanolic Extract High Dose (MEHD-F2) 400 mg/kg b.w.p.o for 28 days

Diabetes was induced in overnight-fasted rats by administering single intraperitoneal (i.p.) injection of freshly prepared control STZ (35 mg/kg) b.w.i.p in 0.1 M citrate buffer (pH 4.5) in a volume of 0.5 ml/kg b.wt. 15 min after i.p. administration of nicotinamide (200 mg/kg) continuously for 2 days. Diabetes was confirmed in the STZ treated rats by measuring fasting blood glucose levels after 48 h of induction. After 24 h of STZ injection, the rats were given 5% w/v of glucose solution (2 ml/kg b.w.) to prevent hypoglycemic mortality. Rats with fasting blood glucose of more than 200 mg/dl were considered as diabetics and they were divided randomly into four different groups. The standard (Glibenclamide 5mg/kg b.w) and herbal formulation were suspended in 1% w/v carboxymethyl cellulose (CMC) and administered once daily through oral gavage for 28 consecutive days. [12]

Investigation parameters

Parameters studied were Body weight, Blood glucose level, food uptake and histology of Kidney and Pancreas.

Histopathology

The pancreas and kidney specimens were immersed in 10% formalin solution for histopathological examination. These tissues were processed, dehydrated in different grades of alcohol, cleared in toluene, and impregnated in molten paraffin wax for specified periods. Processed tissues were embedded in fresh molten paraffin wax and allowed to set. Sections were at 3 µ and dried on a hot plate for 15 min and stained with hematoxylin and 1% aqueous eosin to demonstrate general tissue structure. Stained slides were dehydrated in various ascending grades of alcohol, cleared in xylene, and mounted in Canada balsam. Sections were viewed microscopically using ×10 objective lenses. [13,14]

Statistical Analysis

Results are provided as Mean ± SD (n=6). Results were analyzed statistically using one-way analysis of variance (ANOVA) followed by Bonferroni t-test. $P < 0.05$ was considered as level of significance while comparison between groups.

Results

***In vitro* α-Amylase Inhibitory Assay**

Table 10
α- Amylase Inhibitory Assay of Sample: Acarbose (std.)

Acarbose

Conc.	% inhibition	IC 50 value
20 µg/ml	54.337	
40 µg/ml	57.077	
60 µg/ml	61.643	4.06
80 µg/ml	65.296	
100 µg/ml	71.689	

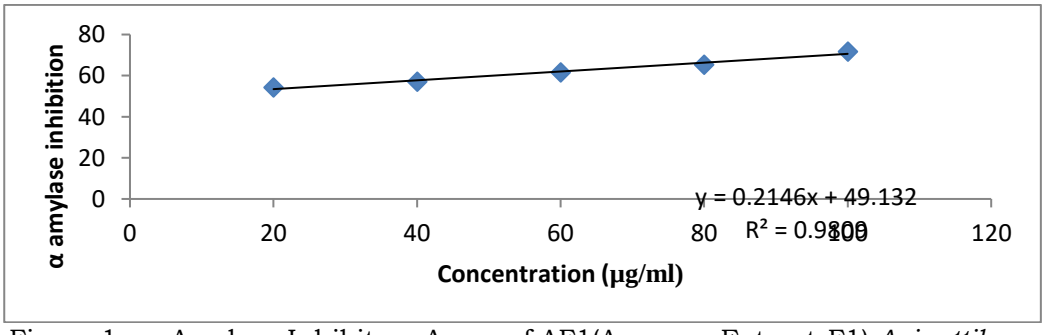

Figure 1. α- Amylase Inhibitory Assay of AF1(Aqueous Extract-F1) *Avipattikara Churna*

Table 11
α- Amylase Inhibitory Assay of AF1(Aqueous Extract-F1) *Avipattikara Churna*

AF1

Conc.	% inhibition	IC 50 value
20 µg/ml	41.187	28.44
40 µg/ml	60.730	
60 µg/ml	67.304	
80 µg/ml	79.634	
100 µg/ml	84.657	

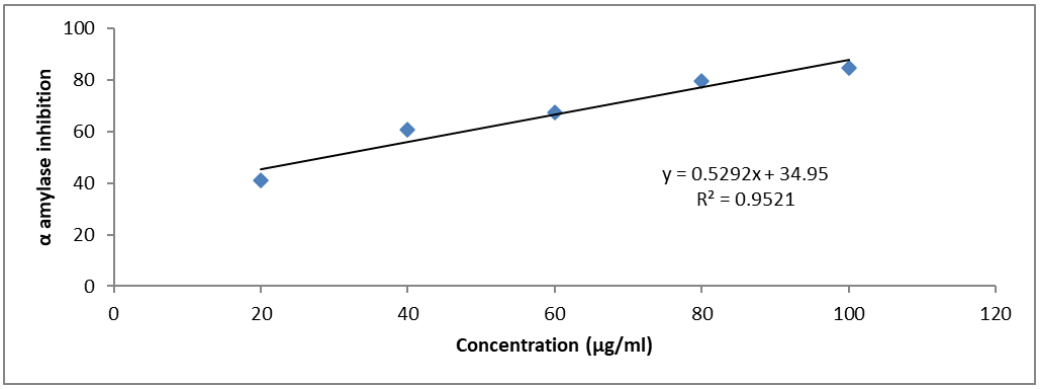

Figure 2. α- Amylase Inhibitory Assay of AF2(Aqueous Extract-F2) *Triphala Churna*

Table 12
α- Amylase Inhibitory Assay of AF2(Aqueous Extract-F2) *Triphala Churna*

AF2

Conc.	% inhibition	IC 50 value
20 µg/ml	31.963	55.66
40 µg/ml	42.100	
60 µg/ml	53.150	
80 µg/ml	63.379	
100 µg/ml	70.045	

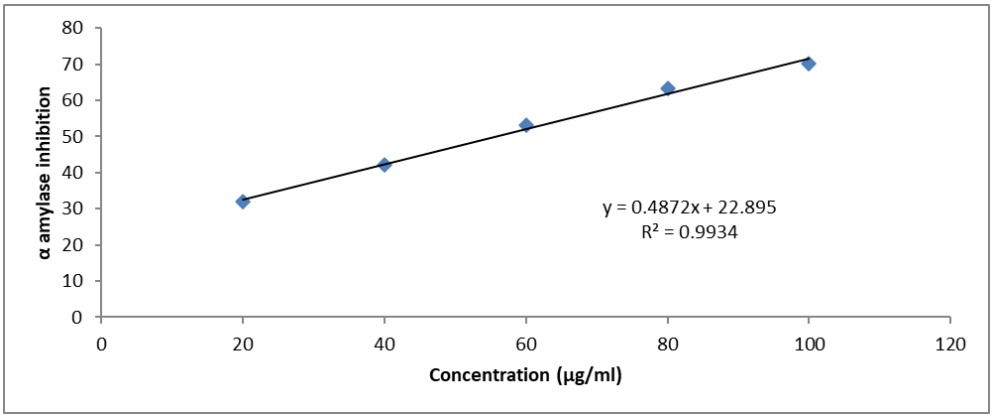

Figure 3. α- Amylase Inhibitory Assay of MF1(Methanolic Extract-F1) *Avipattikara Churna*

Table 13

α- Amylase Inhibitory Assay of MF1(Methanolic Extract-F1) *Avipattikara Churna*

MF1

Conc.	% inhibition	IC 50 value
20 µg/ml	50.68493	
40 µg/ml	56.34703	
60 µg/ml	63.65297	21.53
80 µg/ml	70.68493	
100 µg/ml	79.72603	

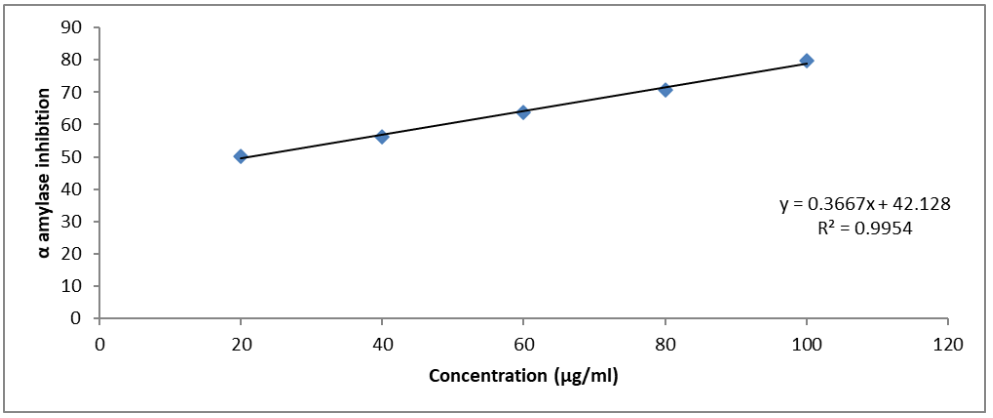

Figure 4. α- Amylase Inhibitory Assay of MF2(Methanolic Extract-F2) *Triphala Churna*

Table 14

α- Amylase Inhibitory Assay of MF2(Methanolic Extract-F2) *Triphala Churna*

MF2

Conc.	% inhibition	IC 50 value
20 µg/ml	44.018	
40 µg/ml	48.401	

60 µg/ml	56.347	41.22
80 µg/ml	62.465	
100 µg/ml	67.214	

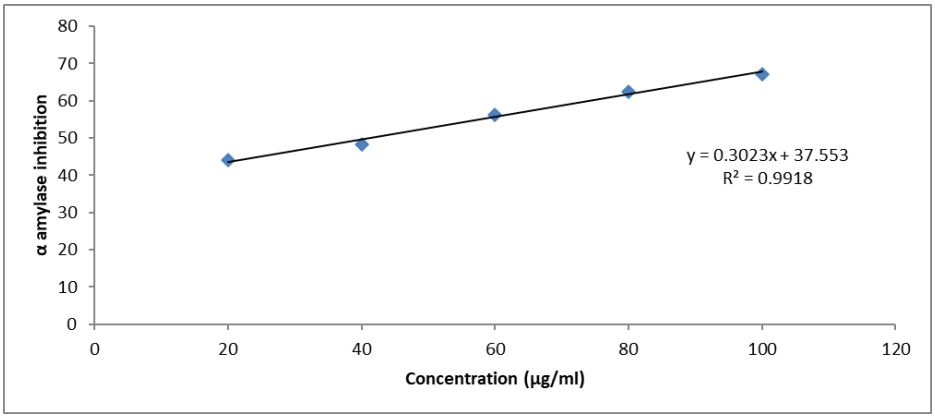

Figure 5. α- glucosidase Inhibitory Assay of Acarbose (std.)

***In vitro* α-glucosidase inhibitory activity**

Table 15
α- glucosidase Inhibitory Assay of Acarbose (std.)

Acarbose

Conc.	% inhibition	IC 50 value
20 µg/ml	54.329	
40 µg/ml	56.426	
60 µg/ml	60.802	4.92
80 µg/ml	66.453	
100 µg/ml	70.829	

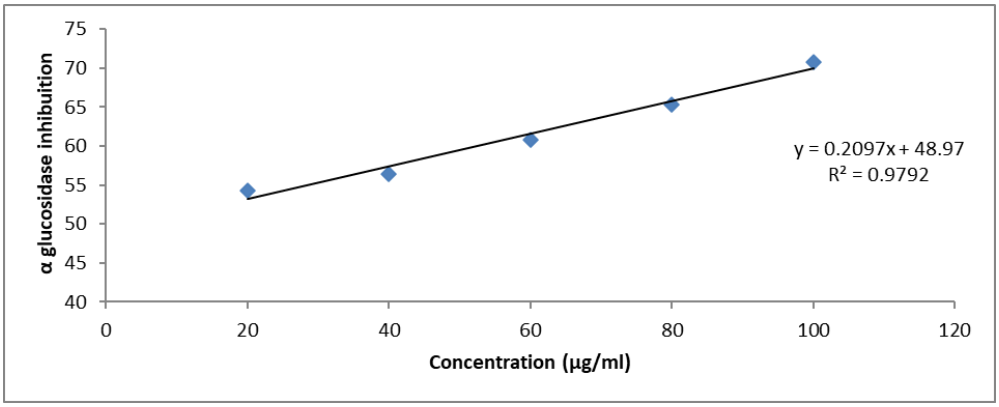

Figure 6. α- glucosidase Inhibitory Assay of AF1 (Aqueous Extract-F1) *Avipattikara Churna*

Table 16
α- glucosidase Inhibitory Assay of AF1 (Aqueous Extract-F1) *Avipattikara Churna*

AF1

Conc.	% inhibition	IC 50 value
20 µg/ml	45.662	36.61
40 µg/ml	50.958	
60 µg/ml	56.894	
80 µg/ml	59.086	
100 µg/ml	68.858	

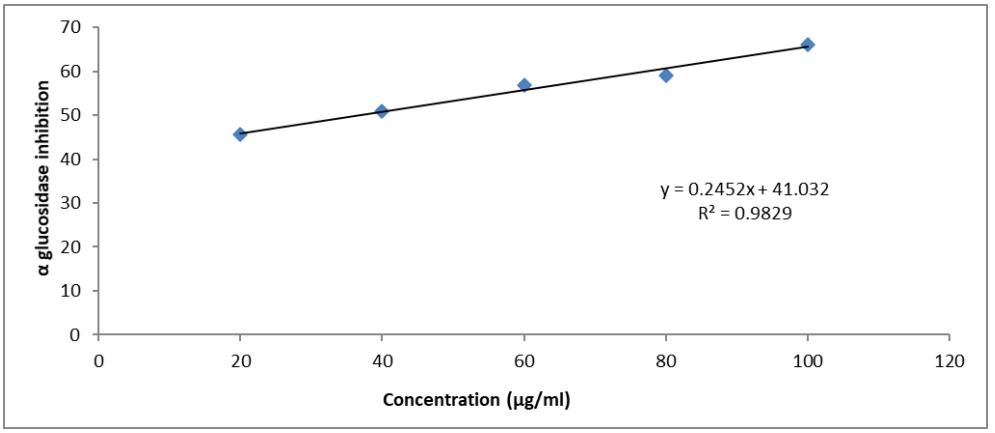

Figure 7. α- glucosidase Inhibitory Assay of AF2((Aqueous Extract-F2) *Triphala Churna*

Table 17
α- glucosidase Inhibitory Assay of AF2((Aqueous Extract-F2) *Triphala Churna*

AF2

Conc.	% inhibition	IC 50 value
20 µg/ml	43.287	47.37
40 µg/ml	48.127	
60 µg/ml	53.881	
80 µg/ml	57.990	
100 µg/ml	61.643	

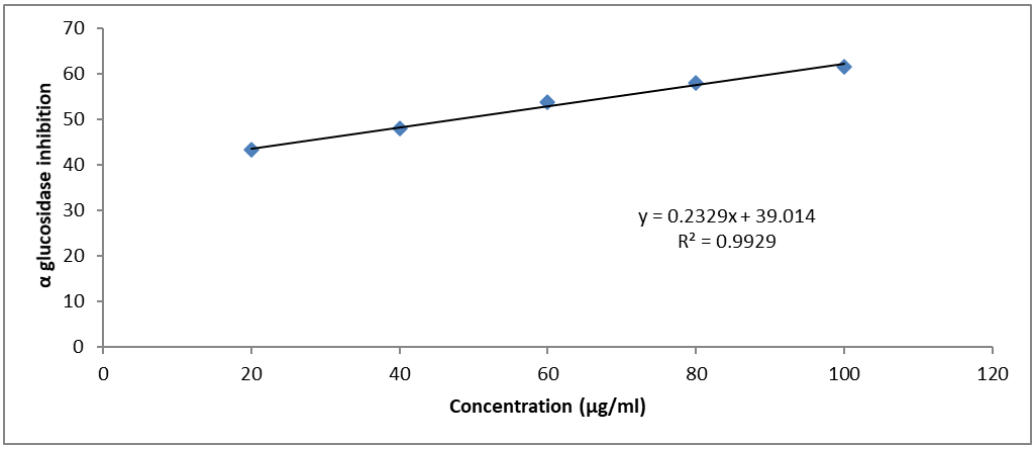

Figure 8. α- glucosidase Inhibitory Assay of MF1(Methanolic Extract-F1) *Avipattikara Churna*

Table 18
α- glucosidase Inhibitory Assay of MF1(Methanolic Extract-F1) *Avipattikara Churna*

MF1

Conc.	% inhibition	IC 50 value
20 µg/ml	48.949	19.69
40 µg/ml	57.625	
60 µg/ml	68.767	
80 µg/ml	74.063	
100 µg/ml	80.182	

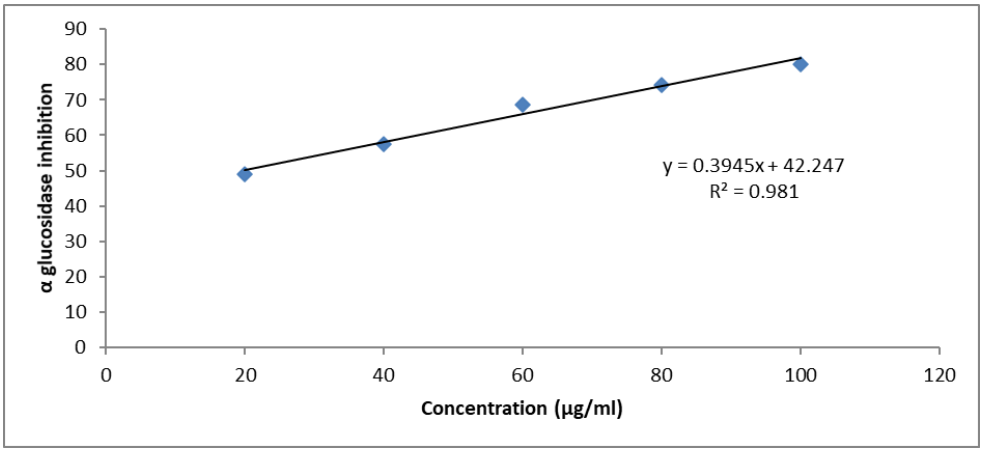

Figure 9. α- glucosidase Inhibitory Assay of MF2((Methanolic Extract-F1) *Triphala*

Table 19
α- glucosidase Inhibitory Assay of MF2((Methanolic Extract-F1) *Triphala Churna*

MF2

Conc.	% inhibition	IC 50 value
20 µg/ml	43.470	
40 µg/ml	46.940	
60 µg/ml	53.333	44.59
80 µg/ml	57.351	
100 µg/ml	63.196	

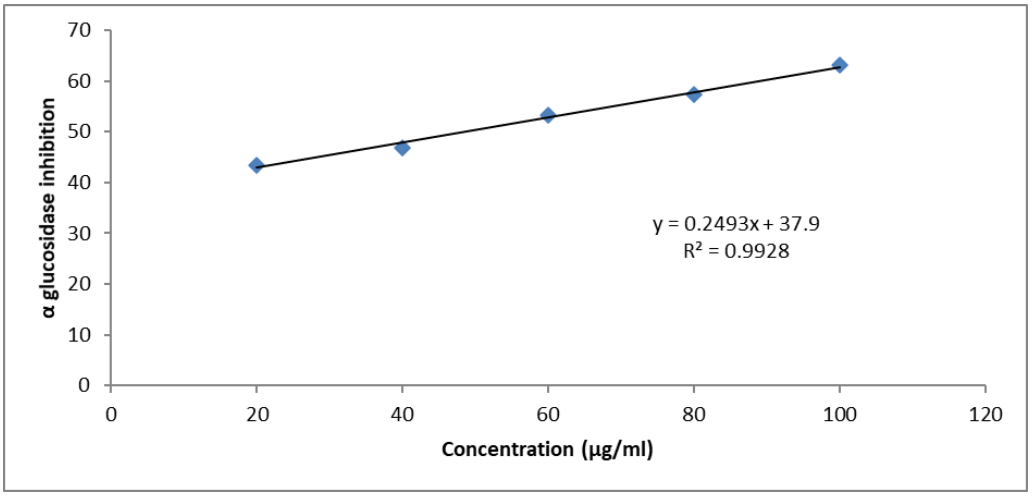

Figure 10. Effect of Test sample AELD-F1,AEHD-F1, AELD-F2,AEHD-F1, MELD-F1, MEHD-F1,MELD-F2,MEHD-F2 on body weight

***In vivo* antidiabetic activity**

Table 20
Effect of Test sample AELD-F1,AEHD-F1, AELD-F2,AEHD-F1, MELD-F1, MEHD-F1,MELD-F2,MEHD-F2 on body weight Variations in body weight in normal control, inducer, standard and Test samples

Group No.	Treatment	O Day	7 Day	14 Day	21 Day	28 Day
I.	Normal	198.49±5.123	202.08±3.357	207.33±3.579	211.05±3.994	214.64±3.687
II.	Positive Control STZ +Nicotinamide	195.41±2.395	191.68±2.539	187.20±4.039	183.98±4.056	179.65±5.262
III.	Std. (5mg/kg b.w)	198.98±3.859	202.46±4.017	207.33±4.080	211.42±4.432	215.17±4.212
IV.	AELD-F1 (200 mg/kg bw)	199.16±2.483	197.46±4.538	201.36±2.971	202.88±3.758	205.77±3.491
V.	AEHD-F1 (400 mg/kg bw)	213.59±8.784	211.59±8.235	219.28±7.974	223.33±8.119	228.87±6.793
VI.	AELD-F2 (200	192.10±4.453	190.17±3.351	191.04±2.624	192.81±3.043	195.88±2.628

	mg/kg bw)					
VII.	AEHD-F2 (400 mg/kg bw)	201.23±5.862	198.92±5.909	196.86±4.652	200.21±5.236	204.27±4.846
VIII.	MELD-F1 (200 mg/kg bw)	214.67±7.875	211.00±7.700	215.66±6.553	219.87±6.699	225.28±5.880
IX.	MEHD-F1 (400 mg/kg bw)	197.80±1.541	201.63±1.024	205.67±1.650	208.67±2.161	212.37±3.681
X.	MELD-F2 (200 mg/kg bw)	194.86±5.426	192.20±4.297	193.90±4.561	196.49±4.011	199.34±4.683
XI.	MEHD-F2 (400 mg/kg bw	199.91±6.128	197.25±6.479	195.53±7.629	199.55±5.850	204.43±4.801

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ^{NS}P>0.001 compared to the Diabetic control group STZ treated. The body weight of normal control rats (Group I) at the beginning of the experiment (i. e. initial body weights) and end of experiment (28th day) was found with normal increase as per growth. A decrease in body weight was observed in diabetic rats (Group II) as compared to the normal control group (Group I) ($p < 0.05$) at the end of the study.

Table 21
Effect of Test samples AELD-F1, AEHD-F1, MELD-F1, MEHD-F1, MELD-F1, MEHD-F1,MELD-F2,MEHD-F2 on fasting blood glucose level Variations in BGL after 0, 7, 14, 21 and 28 day of treatment period

Group No.	Treatment	O Day	7 Day	14 Day	21 Day	28 Day
I.	Normal	198.49±5.123	202.08±3.357	207.33±3.579	211.05±3.994	214.64±3.687
II.	Positive Control STZ +Nicotinamide	320.45±8.185	350.27±3.078	376.51±7.558	393.45±3.94	406.06±3.208
III.	Glibenclamide	277.27±4.733	170.17±2.606	140.49±2.945	119.55±3.646	102.31±2.607
IV.	AELD-F1 (200 mg/kg bw)	324.65±4.724	236.70±3.300	209.09±11.916	185.60±8.868	128.58±13.363
V.	AEHD-F1 (400 mg/kg	315.97±4.651	267.49±6.183	212.19±3.415	166.59±9.622	125.68±12.080

	bw)					
VI.	AELD-F2 (200 mg/kg bw)	330.78±3.571	281.88±4.314	229.13±2.420	181.47±7.759	167.14±6.621
VII.	AEHD-F2 (400 mg/kg bw)	305.09±9.732	274.58±3.375	223.62±8.952	189.73±4.258	153.99±10.111
VIII.	MELD-F1 (200 mg/kg bw)	299.86±7.450	272.24±4.209	223.20±3.481	164.94±2.759	127.68±3.659
IX.	MEHD-F1 (400 mg/kg bw)	307.85±3.868	238.15±4.486	202.54±7.111	163.29±11.135	111.22±7.997
X.	MELD-F2 (200 mg/kg bw)	326.17± 6.373	251.99±5.196	227.96±12.111	178.92±6.201	149.79±9.612
XI.	MEHD-F2 (400 mg/kg bw	271.76±7.689	254.88±1.599	206.74±3.900	159.57±9.489	131.61±11.170

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ^{NS}P>0.001 compared to the Diabetic control group STZ treated. There was a significant increase in the serum glucose levels of the diabetic STZ control group during the course of the study (406.06±3.208). The STZ control group (Group II) showed increased blood glucose referring the impaired glucose tolerance, however, STZ animals on treatment with standard (Group III), and extracts (Group IV-XI) showed normalization in blood glucose levels with their efficacies and dose (Table -).

Table 22
Food uptake effect of Test samples AELD-F1, AEHD-F1, MELD-F1, MEHD-F1, MELD-F1 ,MEHD-F1,MELD-F2,MEHD-F2, Food uptake in various treatment groups during antidiabetic study

Group No.	Treatment		Food Consumption on day (gm/animal)				
			0	7	14	21	28
	Normal	Mean	11.95	12.88	13.93	15.87	16.97
	Positive Control STZ +Nicotinamide	Mean	11.45	12.85	13.15	15.38	16.72
	Glibenclamide	Mean	12.55	15.25	17.85	18.98	19.90
	AELD-F1 (200 mg/kg bw)	Mean	12.85	13.91	14.85	15.95	17.25
	AEHD-F1 (400	Mean	11.67	13.35	14.25	16.55	18.15

	mg/kg bw)						
	AELD-F2 (200 mg/kg bw)	Mean	12.11	12.67	13.23	13.89	14.56
I.	AEHD-F2 (400 mg/kg bw)	Mean	11.71	12.22	13.45	14.23	15.11
II.	MELD-F1 (200 mg/kg bw)	Mean	12.24	14.15	15.87	16.91	18.11
III.	MEHD-F1 (400 mg/kg bw)	Mean	12.67	13.87	15.79	17.28	19.18
IV.	MELD-F2 (200 mg/kg bw)	Mean	11.47	12.39	13.77	14.82	15.99
V.	MEHD-F2 (400 mg/kg bw	Mean	12.33	12.89	13.48	14.88	15.69

Values are expressed as MEAN±SD at n=6

Histopathology of Kidney

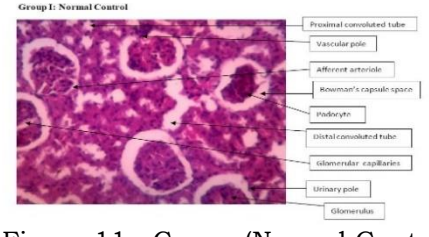

Figure 11 : Group (Normal Control)

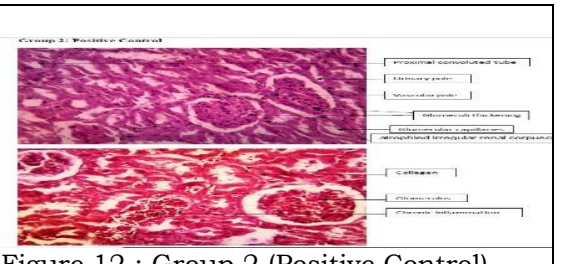

Figure 12 : Group 2 (Positive Control)

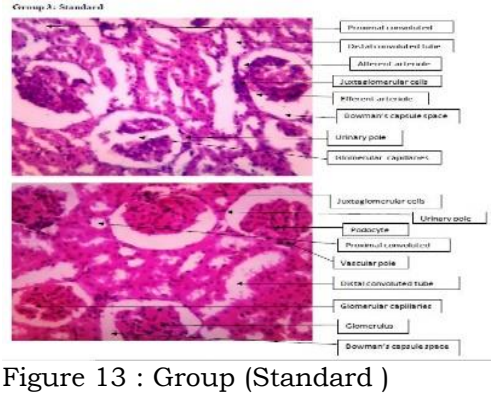

Figure 13 : Group (Standard)

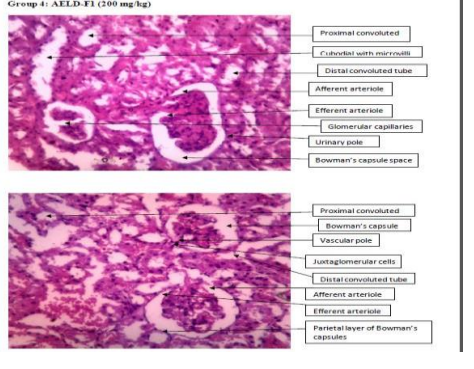

Figure 14 : Group 4 (AEHD F1 200mg/kg)

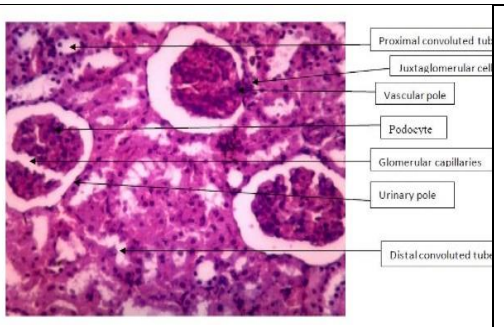

Figure 15 : Group 5 (AEHD F1 400mg/kg)

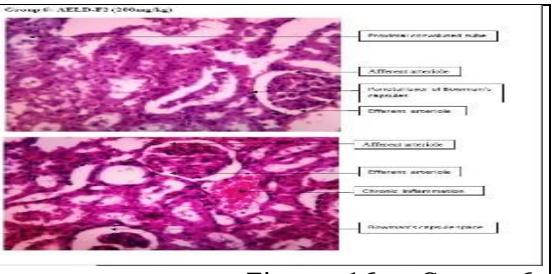

Figure 16 : Group 6 (AELD-F2 200mg/kg

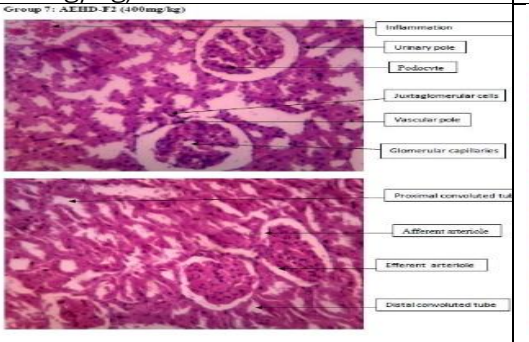

Figure 17 : Group 7 (AEHD-F2 400mg/kg)

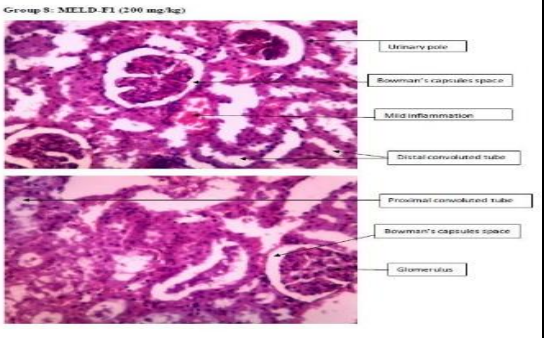

Figure 18 : Group 8 (MELD-F1 200mg/kg)

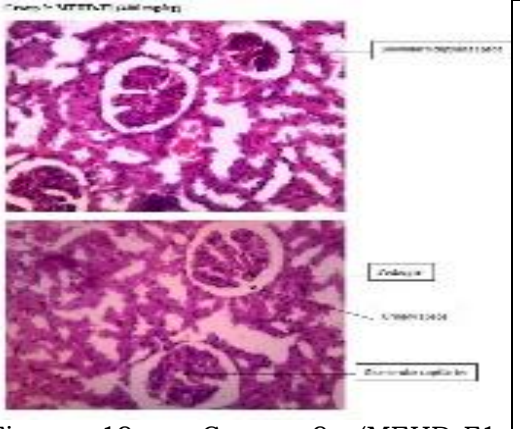

Figure 19 : Group 9 (MEHD-F1 400mg/kg)

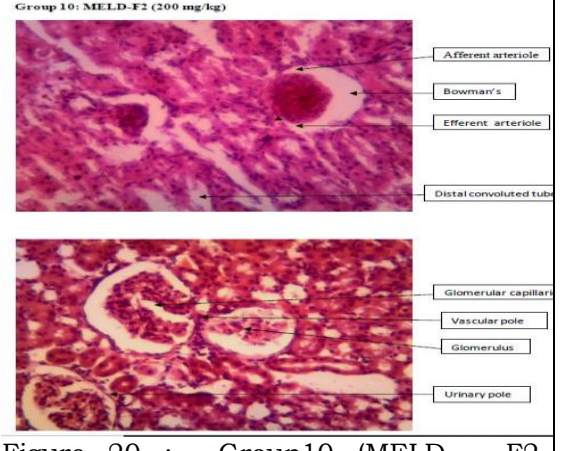

Figure 20 : Group10 (MELD – F2 200mg/kg)

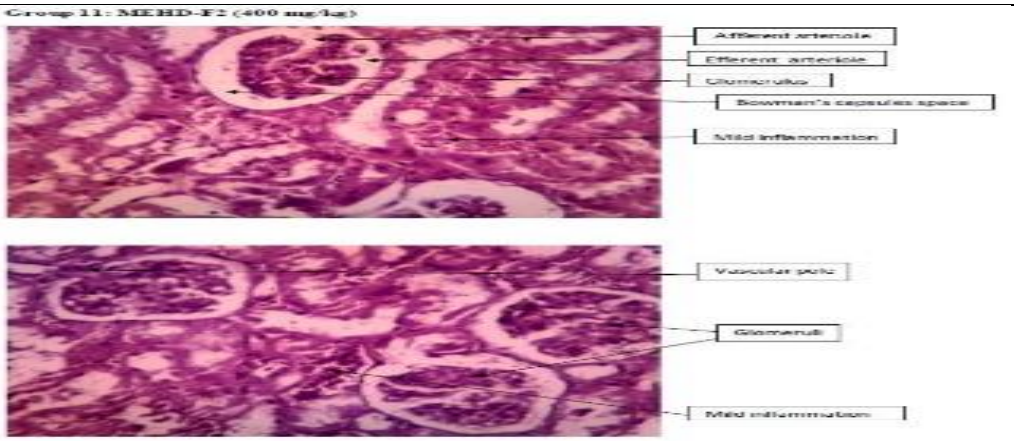

Figure 21 : Group 11 9MEHD-F2 400mg/kg)

Histopathology of Pancreas

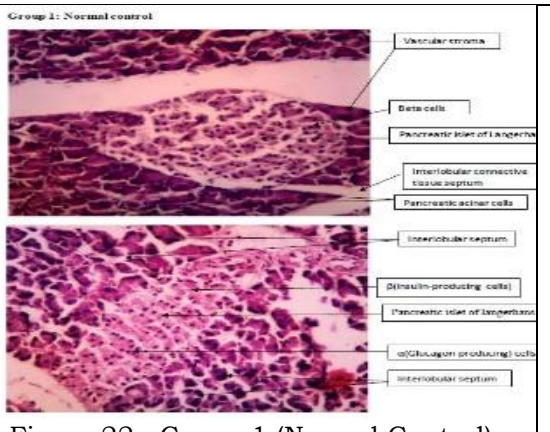

Figure 22 : Group 1 (Normal Control)

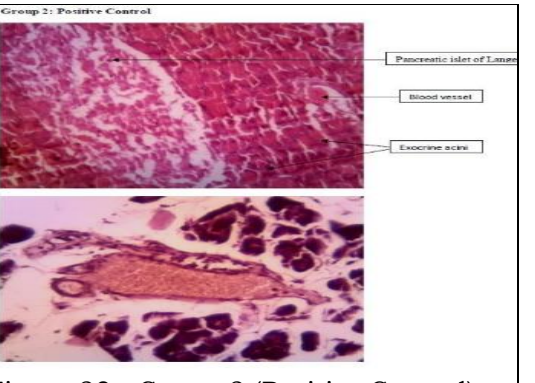

Figure 23 : Group 2 (Positive Control)

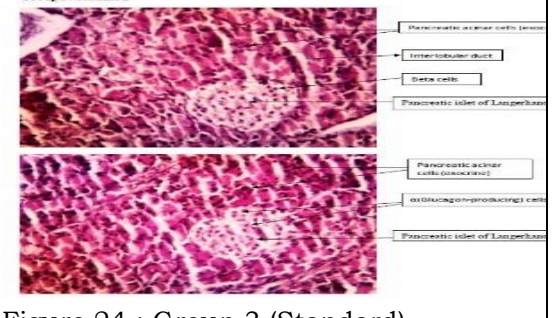

Figure 24 : Group 3 (Standard)

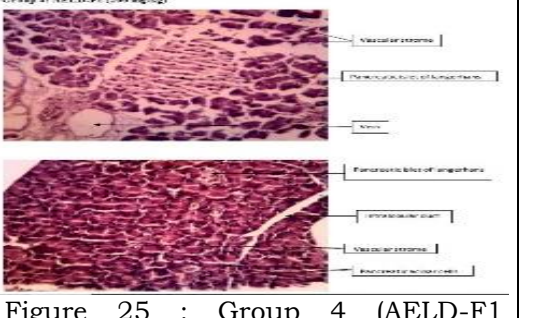

Figure 25 : Group 4 (AELD-F1 200mg/kg)

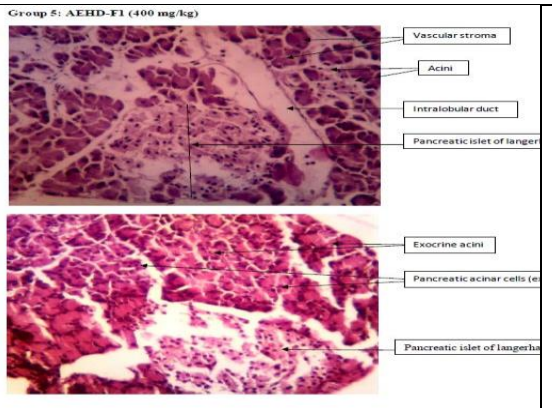

Figure 25 : Group 5 (AEHD-F1 400mg/kg)

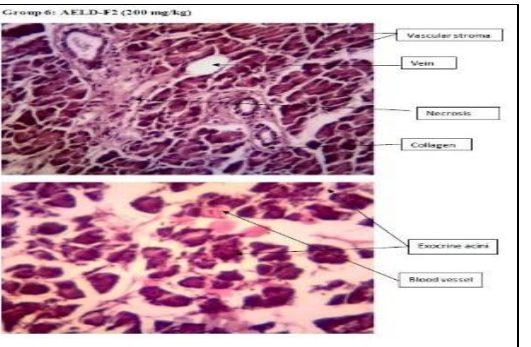

Figure 26 : Group 6 (AELD-F2 200mg/kg)

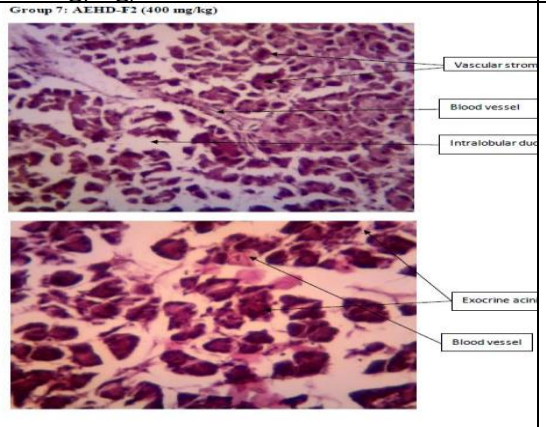

Figure 27 : Group 7(AEHD-F2 400mg/kg)

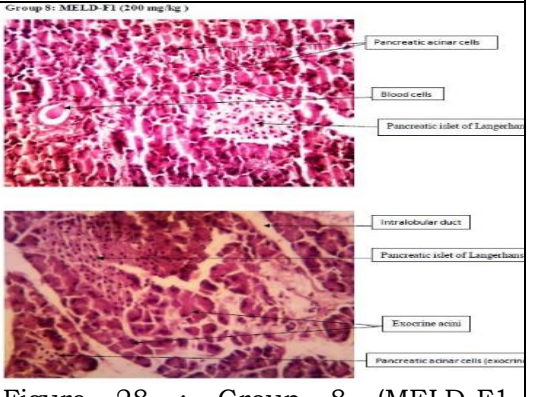

Figure 28 : Group 8 (MELD-F1 200mg/kg)

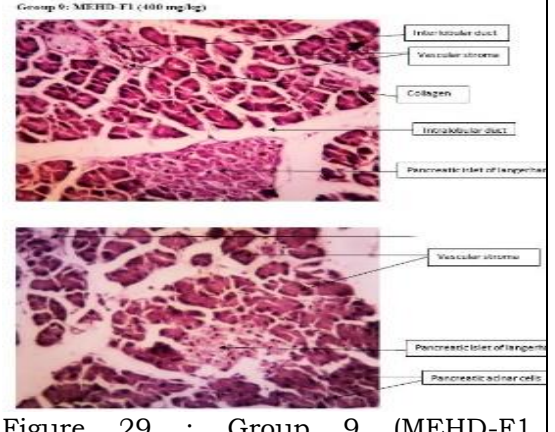

Figure 29 : Group 9 (MEHD-F1 400mg/kg)

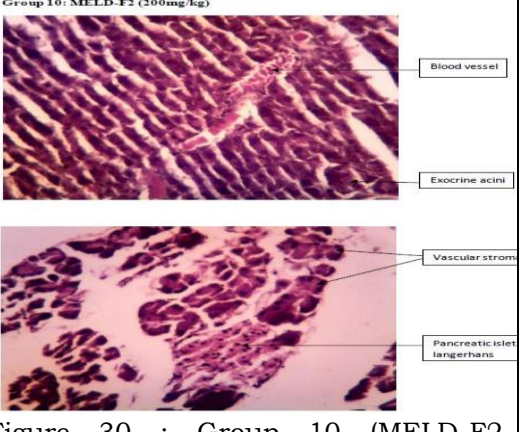

Figure 30 : Group 10 (MELD-F2 200mg/kg)

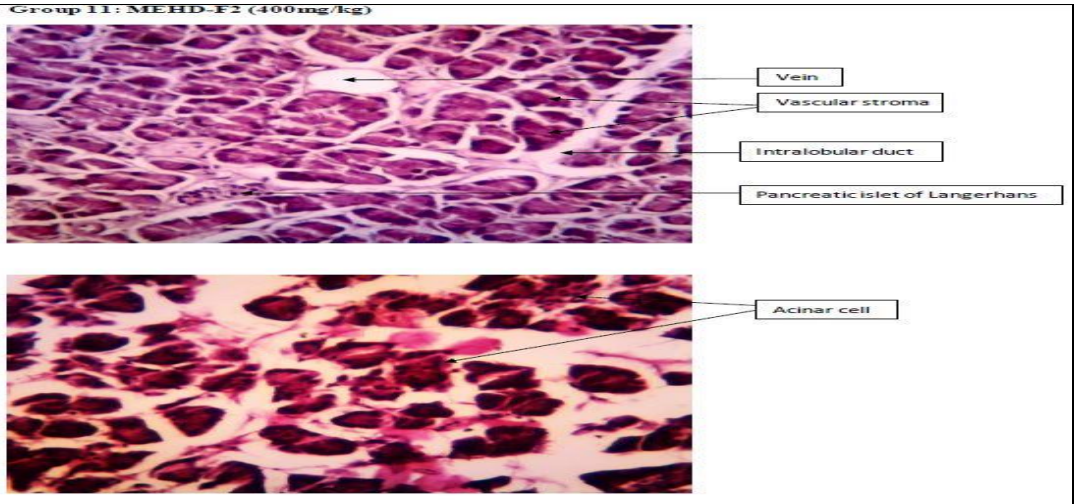

Figure 31 : Group 11 (MEHD-F2 400mg/kg)

Toxicity study of polyherbal formulation

Acute toxicity studies did not show any mortality up to 2000 mg/kg given as single oral administration. Hence, the study was carried out at the dose levels of 200 and 400 mg/kg.

In-Vitro Antidiabetic activity of the Ayurvedic Polyherbal Formulations

During In-vitro analysis for α-amylase and α-glucosidase against Acarbose as standard the Ayurvedic formulations (F1 and F2) showed significant inhibitory effect. [Table-10-19]

In-Vivo Antidiabetic activity of the Ayurvedic Polyherbal Formulations

In the whole of the study, the diabetic animals showed significant reduction in body weight when compared to the control group animals. However, the *Avipattikara Churna* and *Triphala Churna* and Glibenclamide inhibited the diabetes-induced body weight reduction [Table- 20]. Diabetic control animals showed severe hyperglycemia compared to normal animals. The mean blood glucose level in the diabetic control group on day 0 was 320.45±8.185 mg/dl and on day 28 was 406.06±3.208 mg/dl. It was observed that the standard drug Glibenclamide lowered the blood glucose level significantly, bringing it back to near normal level, whereas the *Avipattikara Churna* and *Triphala Churna* at 200 mg/kg and 400 mg/kg significantly ($P < 0.001$) decreased the fasting blood serum glucose level in the diabetic rats on 7th, 14th, and 21st days and 28th days as compared to the diabetic control group. The results are presented in [Table - 21].Diabetic animals showed significant decrease in food intake when compared to the treated groups as shown in [Table-22].

Histopathology results

The histopathologic analysis of kidney and pancreas revealed severe congestion, huge decrease in the number of islets of Langerhans and β cells, and fibrosis and inflammatory cell infiltration into the islets of Langerhans in STZ+ Nicotinamide.induced hyperglycemic rats. In Group 1: Normal control pancreas closely packed by acinar cells and arranged into small lobules. Lobules separated by interlobular connective tissue septa. The islet cells are seen interspersed between the acinar cells. The islets appeared lightly stained than the surrounding acinar cells. Group 2: STZ treated group, distorted islets of Langerhans with necrotic changes and reduced cell number. Less number of beta cells. The acinar cells were swollen. Interlobular ducts were lined with flattened epithelium was observed. Group 3: Standard treated group (Glibenclamide 5mg/kg/b.w), showed slides recovered the general architecture. Most exocrine acini were properly with cytoplasm and cell. Normal interlobular and intralobular duct were observed. Group 4-5 treated with AELD-F1 and AEHD-F1 (200 and 400 mg/kg respectively) group, where slides recovered the general architecture. Normal interlobular and intralobular duct were observed with acinar cells. Group 6-7 treatment group (AELD-F2 and AEHD-F2 200 and 400 mg/kg) represent vascular stroma, necrosis, absence of pancreatic beta cells. Group 8-9(MELD-F1 and MEHD-F1) represents the normal histology of islet of Langerhans with nuclei and interlobular duct and acinar cells. Group 10-11(MELD-F2 and MEHD-F2) slides represent the necrotic changes and reduced cell number. Less number of beta cells, swollen acinar cells. Interlobular ducts were lined with flattened epithelium. Magnification, 10X. [Figure:20 and 21]

Hematoxylin and eosin staining of kidney sections from diabetic rats with and without treatment. Group 1: Normal group: Normal glomerulus and tubules of rat. Group 2: STZ + Nicotinamide induced rats with Glomerular thickening, atrophied irregular renal corpuscles with collapsed glomeruli, interstitial fibrosis and hyaline changes, tubules with degenerated epithelial lining. Group 3: Section showing normal glomerular capillary basement membrane thickening. Tubules and interstitium are within normal limits and normal blood vessels. Group 4 and 5: AELD-F1 and AEHD-F1 (200 and 400 mg/kg respectively) Histological structure of glomeruli and arterioles are similar to those observed in the normal group. Group 6 and 7: (AELD-F2 and AEHD-F2) (200 and 400 mg/kg) Glomerular thickening, interstitial fibrosis and degenerated epithelium Group 8 and 9: (MELD-F1 and MEHD-F1) showing glomerulus with normal cellularity and membrane thickness. Group 10 and 11: (MELD-F2 and MEHD-F2) Milder histological alterations of glomeruli and arterioles are observed. The dark blue color represents the nuclei and the pink color represents cytoplasm, fibronectin and red blood cells. Magnification, 10X. While the polyherbal formulation at the dose of 200 mg/kg and 400 mg/kg showed mild obstruction and moderate decrease in the number of islets of Langerhans with normal β cell population, indicating significant amount of recovery. Glibenclamide treatment showed moderate obstruction with moderate decrease in the number of islets of Langerhans and β cells and mild lymphocytic infiltration [Figure:30 and 31]

Discussion

The *Avipattikara Churna* and *Triphala Churna* formulated using the Methanolic extracts and Aqueous Extract of the plants part given in Ayurvedic Formulary of India. The antidiabetic activity of the individual Formulations has been proven. The methanolic and aqueous extract of *Avipattikara Churna* and *Triphala Churna* showed significant antidiabetic activities against STZ induced diabetes. In the Formulations treated groups there was significant improvement in body weight and food intake as compared to diabetic animals. The toxicity studies were carried out as per the OECD guidelines. The Ayurvedic formulations did not show any mortality or adverse event up to 2000 mg/kg. Hence, the study was carried out at the dose levels of 200 and 400 mg/kg. STZ is toxic glycoside obtained from *Streptomyces achromogenes*, a gram positive bacterium. It accumulates in pancreatic β cells via the glucose transporter 2 (GLUT2) and reduces their expression. The alkylating properties of the STZ modify the biological macromolecules, fragment DNA, and destroy the β cells, causing insulin-dependent diabetes.**[15]** In the diabetic control group, severe body weight loss was observed, which may be due to increased muscle wasting and loss of tissue proteins.**[16]** In the present study, the treatment groups showed significant improvement in body weight, which indicates the Ayurvedic formulations efficacy. The reduction in glucose levels may be due to increase in plasma insulin levels or enhanced transport of blood glucose in the peripheral tissue.**[17]** Our study gives evidence that the Ayurvedic formulations (*Avipattikara Churna* and *Triphala Churna*) increases the plasma insulin levels and has promising antidiabetic activity.

Histopathology of the pancreas [Figure-1 to 11] of STZ+Nicotinamide[24] diabetic animals showed severe decrease in the number of islets of Langerhans and β cells, with fibrosis and inflammatory cell infiltration into the islets of Langerhans, whereas the treated groups showed the normalization of normal tissue texture.[18] Histopathology of the kidney [Figure-12-23] in the STZ diabetic rats showed severe damage, Glomerular thickening, atrophied irregular renal corpuscles with collapsed glomeruli, interstitial fibrosis and hyaline changes, tubules with degenerated epithelial lining [19,20,21,22]. The thickening of basement membrane in glomerulus and tubules, and the progressive accumulation of extracellular matrix components are undertaken due to an increase in gene expression and protein synthesis such as collagen IV, laminin, and fibronectin [23], whereas the formulations treated group showed the normal tissue. Polyherbal formulation and glibenclamide treatment to the animals reduced the severity of the histopathologic changes caused by STZ.

Conclusions

Our study findings suggests that the antidiabetic effect of the Ayurvedic Formulations (Avipattikara Churna and Triphala Churna) both In-vitro and In-vivo at the dose levels of 200 and 400 mg/kg are significant. The antidiabetic potential of both the formulation is comparable with that of Glibenclamide, which is evidenced by decreased levels of blood glucose, change in body weight, food intake.

Acknowledgement

Authors are thankful to KIET Group of Institutions, Delhi-NCR, Ghaziabad for providing all the facility to carry out the research work.

Funding: NIL

References

1. A.R. V Murthy And R.H. Singh.Concept of Prameha/Madhumeha (Contradictions And Compromises) Ancient Science Of Life, Vol. ix, No.2, October 1989.
2. Ayurvedic Formulary of India, Dept. of AYUSH, Govt. of India.
3. Cheng D, Liang B, Li Y. Antihyperglycemic effect of *Ginkgo biloba* extract in streptozotocin-induced diabetes in rats. Biomed Res Int 2013;2013:162724.
4. Crowley ST, Brownlee M, Edelstein D, et al. Effects of nonenzymatic glycosylation of mesangial matrix on proliferation of mesangial cells. Diabetes 1991;40: 540-7.
5. Daye Cheng,1 Bin Liang,2 and Yunhui Li, Antihyperglycemic Effect of Ginkgo biloba Extract in Streptozotocin-Induced Diabetes in Rats, Hindawi Publishing Corporation BioMed Research International Volume 2013.
6. Fattakhov, N., Normatova, S., Madaminov, S., Tilyakhodzhaeva, G., & Abdulkhakimov, A. (2021). Hirudotherapy as an effective method for treatment of migraine - a disease of unknown etiology. International Journal of Health & Medical Sciences, 4(2), 232-237. https://doi.org/10.31295/ijhms.v4n2.1714
7. Gupta Daksha, Chandrashekar, Richard Lobo, Der Pharmacia Lettre; vol.4 (2): pp.614-619, 2012.
8. Huai H. Ethnomedicinal analysis of toxic plants from five ethnic groups in China. Ethnobot Res Appl 2010;8:169-79.
9. Husain SZ, Malik RN, Javaid M, Bibi S. Ethonobotanical properties and uses of Medicinal plants of Morgah Biodiversity Park, Rawalpindi. Pak J Bot 2008;40:1897-911.
10. Kaushik Anu, Formulation And Evaluation Of Herbal Cough Syrup. European Journal Of Pharmaceutical And Medical Research, 2016,3(5), 517-522.
11. Kuo-Ping Shen ,Hui-Li Lin , Hsueh-Wei Yen , Su-Ling Hsieh ,, Li-Mei An , Bin-Nan Wu Eugenosedin-A improves glucose metabolism and inhibits MAPKs expression in streptozotocin/nicotinamide-induced diabetic rats Kaohsiung Journal of Medical Sciences (2018) 34, 142-149
12. Mauer SM, Steffes MW, Ellis EN, et al. Structural functional relationships in diabetic nephropathy. J Clin Invest 1984; 74: 1143-55.
13. Mohsen Pourghasem, Hamid Shafi, Zahra Babazadeh, Histological changes of kidney in diabetic nephropathy, Caspian J Intern Med 2015; 6(3): 120-127
14. Osterby R, Hartmann A, Nyengaard JR, Bangstad HJ. Development of renal structural lesions in type-1 diabetic patients with microalbuminuria. Observations by light microscopy in 8-year follow-up biopsies. Virchows Arch 2002; 440: 94-101.
15. Parasuraman S. Toxicological screening. J Pharmacol Pharmacother 2011;2:74-79.

16. Pari L, Saravanan R. Antidiabetic effect of diasulin, an herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats. Diabetes Obes Metab 2004;6:286-92
17. Pourghasem M, Nasiri E, Shafi H. Early Renal Histological Changes in Alloxan-Induced Diabetic Rats. Int J Mol Cell Med 2014; 3: 11-15.
18. Praveen.K.Dixit,K.Nagarajan,Sokindra Kumar,. Studying the Anti-Diabetic Basis of Ayurvedic Formulations –Avipattikara Churna And Triphala Churna, International Journal of Research in Pharmaceutical Sciences, 11(4), 5589-5595,July,2020
19. Rajendran Ramesh Petchi1, Chockalingam Vijaya1, Subramani Parasuraman2, Antidiabetic Activity of Polyherbal Formulation in Streptozotocin – Nicotinamide Induced Diabetic Wistar Rat, Journal of Traditional and Complementary Medicine Vo1. 4, No. 2, pp. 108-117,2014.
20. Samsulrizal Nurdiana , Yong Meng Goh , Hafandi Ahmad , Sulaiman Md Dom , Nur Syimal'ain Azmi, Noor Syaffinaz Noor Mohamad Zin and Mahdi Ebrahimi, Changes in pancreatic histology, insulin secretion and oxidative status in diabetic rats following treatment with Ficus deltoidea and vitexin, Nurdiana et al. BMC Complementary and Alternative Medicine (2017) 17:290
21. *Shaifali Gurjar*, *Anuradha Pal*, *Suman Kapur*. Triphala and its constituents ameliorate visceral adiposity from a high-fat diet in mice with diet-induced obesity, Altern Ther Health Med Nov-Dec 2012;18(6):38-45
22. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001;50:536-46.
23. Talent Chipiti, Mohammed Auwal Ibrahim, [...], and Md. Shahidul Islam, In vitro α-amylase and α-glucosidase inhibitory effects and cytotoxic activity of Albizia antunesianaextracts Pharmacognosy Magazine,2015 Oct,11(2)S231-S236
24. Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, *29*(3), 550–561. https://doi.org/10.1002/cae.22202
25. Wilcox G. Insulin and Insulin Resistance. Clin Biochem Rev 2005;26:19-39.
26. Ziyadeh FN, Hoffman BB, Han DC, et al. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-β antibody in db/db diabetic mice. Proc Natl Acad Sci U S A 2000; 97: 8015-20.