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Synergistic Antidiabetic Activity of Polyherbal Formulation

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ABSTRACT

The aim & objective of the research is to evaluate the synergistic anti diabetic activity of polyherbal drugs containing *Gymnema Sylvestre* (Leaves),*Trigonella Foenum-Gr* (Leaves), *Tinospora Cardifolia* (Stems), *Azadirachata Indica* (Leaves), *Cinnamomum Zeylanicum* (Stem Barks), *Syzygium Jambolana* (Fruits) & *Nardostachys Jatamansi* (Roots) which are well-known medicinal plants available throughout India and they are commonly used for the treatment of various diseases. All the herbal drugs were collected from herbal shop (Grovel Drugs & Chemicals Pvt Ltd.Hyderabad). 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 herbal drugs were within the limits. The developed polyherbal formulation is subjected for extraction with ethanol (90%) by cold maceration & soxhelation method to get ethanolic extract. The polyherbal ethanolic extract is evaluated for its antidiabetic and antioxidant activity. Screening of preliminary phytochemical investigation of polyherbal ethanolic extract shows the presence of large amounts of phenolic, tannins & flavonoid compounds which exhibits the highest antioxidant and free radical scavenging and also inhibited lipid peroxidation.

The polyherbal extract shows synergistic effect of antidiabetic activity and it may be used as an alternative remedy for the treatment of diabetes mellitus.

Key words: Polyherbal drugs, Antidiabetic, Antioxidant, Flavonoids, Ethanol, etc.

1.Introduction

Correspondingly to conventional medicines, the indications of folk HMs are diverse, being employed for the treatment of a wide range of diseases. The indications spread from simple health conditions such as cold, pain, surface wounds to serious conditions such as psychosis, diabetes, malaria, sickle cell disease, tuberculosis, cancer, hypertension, infertility, and so on. In certain communities, HM is a major component of the primary healthcare. Indeed, up to 80% of the rural world population use herbal-based traditional medicines for most of their healthcare[1].

Oxidative stress is the major driving factor responsible for the initiation and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes[2]. The condition is brought by excessive generation of free oxygen and nitrogen species or their inefficient quenching in the cell. On the other hand, endogenous sources of free radicals include the electron transfer chain reactions in the mitochondria, xanthine oxidase pathway, during disease states such as inflammation, ischemia and reperfusion injury[4]. The body possesses a complex antioxidant defense system, comprising of enzymatic and nonenzymatic pathways, which in the normal physiologic state, maintain a steady equilibrium between prooxidants and antioxidants, thereby ensuring well-being[2]. However, during disease states, the endogenous antioxidant systems are overwhelmed, leading to accumulation of excessive free radicals, which inturn cause oxidative stress-associated damage to cellular machinery, as implicated in various diseases[5].

Conventionally, oxidative stress is managed using various synthetic antioxidant compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG). Despite their usage, these synthetic antioxidant compounds have been associated with undesirable effects[6]. For instance, BHT and BHA cause hepatotoxicity and have been demonstrated to be carcinogenic. Additionally, synthetic antioxidants are inaccessible, unaffordable, and labile, thus limiting their utilization[4].Therefore, due to the profound consequences of oxidative stress and the drawbacks of synthetic antioxidants, the need for alternative antioxidants, which are safer, easily accessible, and potent, are warranted[7], hence the current study. Considering the available alternative and complementary strategies, medicinal plants stand a better chance of providing potent, safer, affordable, and easily accessible therapies for oxidative stress-related maladies[8]. Medicinal plants contain various secondary metabolites, which have demonstrated a wide spectrum of pharmacologic activities. Antioxidants properties of plants have been demonstrated to play a protective role in the body against diseases, since their consumption lowers the risk of cancer, heart disease, hypertension, dementia, and stroke [9]. The major groups of phytochemicals that contribute to antioxidant capacity of plants include polyphenols and vitamins (A, C, and E). Phenolic compounds of plants are hydroxylated derivatives of benzoic acid and cinnamic acids, which possess antioxidant and anticarcinogenic effects[4]. & they include phenols, flavonoids, coumarins, tannins, and anthocyanidins. & theese phytoactive complexes are important in plant defense mechanisms against biotic and abiotic stresses[10]. When plants or plant products rich in these phytoactive principles are consumed, they are deemed to confer the same beneficial effects to humans[9]. For instance, flavonoids have for long been recognized to possess anti-inflammatory, antiallergic, antiviral, immunomodulatory, antiaging, and antiproliferative properties[11]. The search for better alternatives to synthetic antioxidants has triggered a

significant research interest on dietary and medicinal plants that can inhibit, reverse or ameliorate diseases caused by oxidative stress [4, 11]. In this present study, we investigated the in vitro antioxidant activity of the ethanolic extracts of different parts of medicinal herbal drugs belonging to different families.

2.Materials and methods

Table 2.1: List of herbal drugs

S.no	Botanical Name	Common name	Parts used
1	*Gymnema Sylvestre*	Gudmar	Leaves
2	*Trigonella Foenum-Gr*	Fenugreek, Methi	Seeds
3	*Tinospora Cardifolia*	Guduchi	Stems
4	*Azadirachata Indica*	Neem	Leaves
5	*Cinnamomum Zeylanicum*	Cinnamon	Barks
6	*Syzygium Jambolana*	Jamun	Fruits
7	*Nardostachys jatamansi*	Jatamansi, Spikenard	Roots

2.1 Preparation of the formulation[12,13]: All powdered drugs were taken in various proportions (Table 2) and sieved using mesh no. 85. Powdered drugs were sterilized using UV radiation at 60°C. Powdered drugs were homogenized using mortar and pestle. The powdered drugs were mixed in a geometrical ratio with continuous mixing to obtain the desired formulation. The prepared polyherbal formulation is stored in air tight container for further use.

Table 2.1.1: Preparation of polyherbal formulation

S.no	Powder Plant Name	Parts used	Concentration in gms
1	*Gymnema Sylvestre*	Leaves	50
2	*Trigonella Foenum-Gr*	Seeds	50
3	*Tinospora Cardifolia*	Stems	50
4	*Azadirachata Indica*	Leaves	50
5	*Cinnamomum Zeylanicum*	Barks	50
6	*Syzygium Jambolana*	Fruits	50
7	*Nardostachys jatamansi*	Roots	50

2.2 Extraction of polyherbal extract[12,13]:

The polyherbal formulation is extracted by using cold maceration using 90% ethanol. The extract was evaporated using Rota flash evaporator under reduced pressure and low temperature and then on a water bath. The obtained marc is then air dried. The obtained air dried marc were subjected for extraction with ethanol(90%) with the help of Soxhlet apparatus at 50ºC. The solvent was evaporated using Rota flash evaporator under reduced pressure. The dried extract were preserved in refrigerator (4 ºC) for further use. The percentage yield, colour and consistency of the extracts were recorded.

2.3 Preliminary phytochemical investigation of herbal extracts

Preliminary phytochemical investigations of all extracts:

The various extracts of herbal drugs obtained were screened for its chemical constituents with the following tests[14,15].

2.3.1Test for Alkaloids

The small portions Fraction was stirred separately with a few drops of dilute HCl, filtered and filtrates were subjected for the following tests.

a) Dragendorff's Test

Filtrates were treated with dragendorff's reagent (Potassium Bismuth iodide).

Formation of orange-brown precipitate indicates the presence of alkaloids.

b) Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium mercuric iodide).

Formation of cream precipitate indicates the presence of alkaloids.

c) Wagner's Test

Filtrates weretreated with Wagner's reagent (Potassium iodide).

Formation of reddish-brown precipitate indicates the presence of alkaloids.

d) Hager's Test

Filtrates were treated with Hager's reagent (saturated solution of picric acid).

Formation of Yellow precipitate indicates the presence of alkaloids.

2.3.2 Test for Glycosides

a) Modified Borntrager's test (For Anthraquinone glycosides)

About 0.1 g of extract/fraction was boiled with dilute hydrochloric acid for 2 minutes and few drops of ferric chloride solution were added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia was added to the benzene extract and shaken well. Formation of pink colour indicates the presence of anthraquinone glycosides.

b) Test for Cardiac Glycosides (Keller-killiani test)

About 1 g of the extract/fraction was boiled with 10 ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10 ml of water and 5 drops of the solution of lead sub-acetated were added and filtered, evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid. To this 2 ml of ferric chloride solution was added from the side of the test tube carefully and observed. Formation of reddish colour ring at the junction indicates the presence of cardiac glycosides.

c) Legal's Test (For Cardinolides)

Extract/fraction was dissolved in pyridine. Add sodium-nitroprusside solution to it. Formation of pink or red colour indicates the presence of cardiac glycosides.

2.3.3 Test For Saponins

1) About 0.5 g of the extract/fraction was boiled gently for 2 minutes with 20 ml of water and filtered while hot and allowed to cool. 5 ml of the filtrate was taken, dilute with water and shaken vigorously. Frothing occurred indicating the presence of saponins.

2) Liebermann Burchard's Test

To the extract/fraction, a mixture of acetic anhydride and concentrate sulphuric acid (19:1) was dissolved in suitable anhydride solvent; development of violet purple colour, indicting the presence of steroidal-saponins.

2.3.4 Test For Tannins

1) To the extract/fraction of the powdered drug, few drop of ferric chloride was added. Bluish colour indicates the presence of tannins.

Test For Flavonoids

a) Shinoda test

A little amount of extract/fraction was dissolved in alcohol and filtered. The test solution obtained as above was treated with magnesium ribbon and few drops of concentrated hydrochloric acid. Boil for few minutes, characteristic magentacolor is indicates the presence of flavonoids.

b) Alkali test

To the alcoholic solutionof extract/fraction, 10% potassium hydroxide solution was added. Formation of intense yellow colour indicates the presence of flavonoids. This yellow colour becomes colourless on addition of dilute acid.

c) Acid test

To the alcoholic solution of extract/fraction, few drops of sulphuric acid is added. Change indicates presence of flavonoids.

e) Lead acetate test

The alcoholic solution of extract/fraction was treated with few drops of lead acetate solution. Formation of yellow coloured precipitate indicates the presence of flavonoids.

2.3.5 Test For Steroids

a) LiebermannBurchard's Test

The extract/fractionwas dissolved in chloroform and filtered. Glacial acetic acid, one drop of conc. Sulphuric acid was added to the solution. Rose to violet or blue to green color indicates the presence of steroids.

b) Salkowski's Test

To the solution of sample, conc.sulphuric acid was added. Red color indicates the presence of steroids.

2.3.6 Test For Terpenoids

a) Salkowski's Test

Few mg of extract/fraction was added into chloroform and conc.sulphuric acid was added. The yellowish white colour slowly becomes red, indicating the presence of triterpenoids.

b) Liebermann Storch Morawski Test

Few mg of extract/fraction was dissolved in few ml in acetic anhydride and few drops of sulphuric acid were added. Red or blue color produced which indicating the presence of triterpenoids.

c) Hirschorn Test

Extracts were treated with trichloroacetic acid and warmed. Red to yellow colour produced which indicates the presence of triterpenoids.

2.3.7 Tests For Carbohydrates

Extract were dissolved separately in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2ml of concentrated sulphuric acid was added carefully along with the side of the test tubes. Formation of violet ring at the junction indicates the presence of carbohydrates.

b) Benedict's Test

Filtrates were treated with Benedict's reagent and heated in water bath. Formation of orange red precipitates indicates the presence of reducing sugars.

c) Fehling's Test

Filtrates were hydrolyzed with dilute HCl, then neutralized with alkali and heated with Fehling's A and Fehling's B solutions. Formation of red precipitates indicates the presence of reducing sugars.

d) Barfoed's Test

Filtrates were treated with Barfoed's reagent and heated on a water bath. Formation of red precipitate indicates the presence of monosaccharides.

2.3.8 Test for Phenol: About 0.5 g of each of the studied plant extracts was boiled in 5 ml of 70% ethanol in a water bath for 5 minutes and then filtered through Whatman filter paper No. 1. After cooling, 5 drops of 5% ferric chloride were added and mixed. The appearance of a green precipitate indicates the presence of phenols in the sample.

2.3.9 Test for Saponins: About 2 g of each of the studied plant extracts was weighed and dissolved in 5 ml of distilled water. Thereafter, aliquots of 2 ml were taken from each plant extract solution, stirred for 30 seconds, and briskly agitated. &e setups were then allowed to settle for 15 minutes. &e presence of frothing, which persists for over 15 minutes, is an indication of the presence of saponins in the tested sample.

2.3.10 Test for Volatile oils:

Sudan red III test: Thin section of sample + Sudan red III solution gives red color

Tincture alkane test: Thin section of sample + tincture alkane solution gives red color.

2.4 Determination of antidiabetic & antioxidant activities of polyherbal formulation (EEPF)

2.4.1 Experimental pharmacology studies

Wistar albino rats of both sex weighing between 150–200 gms were used for the study. All animals were maintained under standard laboratory conditions [temperature 22±2℃ and humidity 45±5 ℃)] with 12 hours day: 12 hours night cycle. The animals were fed with normal laboratory diet and allowed to drink water *ad libitum*. All the experimental procedures conducted after the approval of Institutional Animal Ethical Committee (IAEC) and CPCSEA guidelines for the use and care of experimental animals **(1567/PO/RE/S/11/CPCSEA)**.

2.4.2 Acute toxicity studies (LD_{50}) [16,17].

The acute toxicity of Etanolic Extract of Polyherbal Formulation (EEPF) was determined by using Wistar rats (150-200gms/kg b w). The animals were fasted for 24 hrs prior to the experiment and up and down procedure (OECD guideline no. 425) method of CPCSEA was adopted for acute toxicity studies. A maximum dose up to 5000 mg/kg has been tested for any mortality. From this, four doses are selected for the study, 200 mg/kg, 300mg/kg,400mg/kg & 500mg/kg b w which are $1/10^{th}$ of the LD_{50} values.

2.4.3 Antidiabetic activity:

2.4.3.1 Streptozotocin induced diabetes: [18]

In the present investigation, a total of 42 rats (36 diabetic surviving rats and 6normal rats) were taken and divided into seven groups of 6 rats each.

Procedure: Wistar rats of either sex weighing between 150-200 gms were divided into seven groups of six rats in each.

Group I: Animals were maintained as normal control, which was given with distilled water only.

Group II: Animals were received Streptozotocin (55 mg/kg i.p).

Group III: Animals were treated with standard drug glibenclamide (600 µg/kg b w) which served as standard[19].

GroupIV: Animals were treated with EEPF (200 mg/kg b w oral).

Group V: Animals were treated with EEPF (300 mg/kg b w oral).

Group VI: Animals were treated with EEPF (400 mg/kg b w oral).

Group VII: Animals were treated with EEPF (500 mg/kg b w oral).

Three days after stz injection, rats screened for diabetes having glycosuria and hyperglycemia with blood glucose level more than 250 mg/dl was taken for the study. All animals were allowed free access to water and pellet dietand maintained at room temperature in plastic cages. Treatment was given for 21 days. The animals were sacrificed at the end of the experimental period of 21 days. blood collected by retro orbital puncture and biochemical parameters like body weight, Serum Glucose, SGOT, SGPT, HDL, Triglycerides, Cholesterol, Creatinine and Total Proteins were estimated. The animals were sacrificed by overdose of ether and autopsied. Pancreas from all animals were removed, washed with ice-cold saline, small piece of liver tissue was collected and preserved in 10% formalin solution for histopathological studies. Pancreas of some animals were homogenized with ice-chilled 10% KCL solution and centrifuged at 2000 rpm for 10 minutes. Then the supernatant liquid was collected and the antioxidant parameters like

Catalase, Super oxide Dismutase, LPO and GPx were estimated.

2.4.3.2 Physical Parameters: Body weight was taken every week.

2.4.3.3 Biochemical parameters:

a. Estimation of glucose: (GOD/POD method) (Span Diagnostics Ltd., India) [20]

b. Estimation of Serum Triglycerides (Enzymatic method)[21]

c. Estimation of HDL cholesterol[22-26]

d. Estimation of Serum ALP[27] **: (pNPP kinetic method)**

e. Estimation of serum Creatinine[28,29]**: (Mod jaffe's Kinetic method)**

f. Estimation Of Serum SGPT (UV- Kinetic method)[30]

g. Estimation of Serum SGOT (UV- kinetic method)[30]

h. Estimation Of Serum Total Proteins[31]**:**

2.4.4 In-vitro antioxidant activity

Preparation of Pancreas homogenate: Pancreatic tissue were homogenized in KCl [10 mM] phosphate buffer (1.15%) with EDTA (pH 7.4) and centrifuged at 12000 rpm for 60 min. The supernatant was used for assay of the marker enzymes (GPx, SOD, CAT, LPO) and protein estimation.

2.2.4.1 Lipid Peroxidation activity[32]

2.2.4.2 Glutathione peroxidase activity [33]

2.2.4.3 Super Oxide dismutase activity [34]

2.2.4.4 Catalase activity activity[35]

2.2.4.5 Total protein content activity[36]

3.Results and discussion

3.1 Percentage yield, colour and consistency of polyherbal extracts

Table 3.1: Percentage yield, colour and consistency of polyherbal extracts

S.No.	Extracts	Colour in day light and consistency	% Yield
1	EEGS	Semisolid dark brown	18.17
2	EETF	Semisolid Golden brown	15.55
3	EETC	Semisolid Light Yellow	32.98
4	EEAI	Semisolid greenish brown	29.67
5	EECZ	Semisolid brown	11.89
6	EESJ	Semisolid light brown	11.24
7	EENJ	Semisolid dark brown	28.56

3.2 Preliminary phytochemical investigation of herbal extracts

Table 3.2.1: Preliminary phytochemical constituents present in ethanolic polyherbal extracts.

S.no	Phyto Constituents	EEGS	EETF	EETC	EECZ	EESJ	EENJ	EEAI
1	Alkaloids	+	+	+	+	+	+	+
2	Glycosides	+	+	+	+	+	+	+
3	Flavonoids	+	+	+	+	+	+	+
4	Tannins	-	-	+	+	+	+	+
5	Steriods	-	-	-	-	-	+	-
6	Terpenoids	+	+	+	+	+	+	+
7	Carbohydrates	-	-	-	-	-	-	-
8	Proteins	-	-	-	-	-	-	-
9	Phenols	+	+	+	_	_	+	_
10	Saponins	+	_	+	-	-	+	_
11	Volatile Oils	_	+	_	+	_	_	+
12	Gums	_	_	_	_	_	_	_

+ indicates positive, - indicates negative result

Table 3.2.2: Preliminary phytochemical constituents present in ethanolic extract of polyherbal formulation.

S.No	Chemical Constituent	EEPF
1	Alkaloids	+
2	Glycosides	+
3	Flavonoids	+
4	Tannins	+
5	Steroids	-
6	Terpenoids	+
7	Carbohydrates	-
8	Proteins	-
9	Saponins	+
10	Gums	+
11	Phenols	+
12	Volatile oils	+

+ indicates positive, - indicates negative result

3.3 ANTIDIABETIC ACTIVITY

3.3.1 Streptozotocin Induced Diabetes

3.3.1.1 Body weight:

Intraperitoneal injection of streptozotocin (55 mg/kg) in adult rats produced cardinal signs of type I diabetes i.e., loss of body weight, polyphagia and polydipsia. Glucosuria and polyuria observed in these animals persisted throughout the period of three weeks. Chronic treatment with polyherbal formulations prevented loss of body weight in diabetic rats.

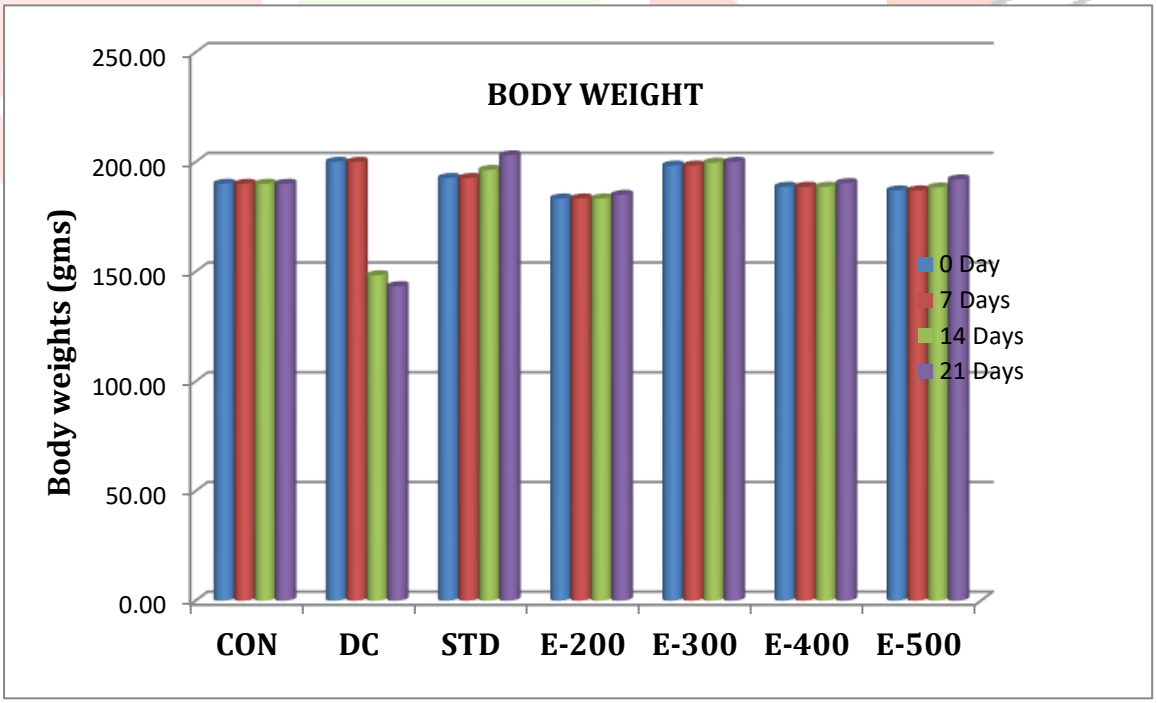
After 21 Days, diabetic rats exhibited significantly lower Body weight (143.33±12.02) as compared to those of control rats (190±5.77). Treatment with polyherbal formulation could significantly elevate the reduced Body weight. EEPF-200 (185±2.88), EEPF-300 (200±7.63**), EEPF-400 (190.33±8.81*) and EEPF-500 (192±13.54*). Glibenclamide treated rat's serum glucose level was (203±2.66**).

Table 3.3.1.1: Effect of polyherbal formulation (EEPF) on body weight in diabetic rats.

Groups	Treatment	Dose	Body weight in gms			
			0th day	7th day	14th day	21th day
I	Control	10 ml/kg	190±5.77	190±5.77	190±2.88	190±5.77
II	Diabetic control stz	55 mg/kg	200±2.88	200±2.88	148.33±7.26	143.33±12.02
III	Glibenclamide	600 µg/kg	192.66±4.41	192.66±4.41	196.33±4.41*	203±2.66**
IV	EEPF	200 mg/kg	183.32±4.41	183.32±4.41	183.32±4.41	185±2.88
V	EEPF	300 mg/kg	198.33±9.28	198.33±9.28	199.66±8.81*	200±7.63**
VI	EEPF	400 mg/kg	188.66±8.81	188.66±8.81	188.66±8.81	190.33±8.81*
VII	EEPF	500 mg/kg	187±12.58	187±12.58	188.33±11.01	192±13.54*

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test.Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$,*** represents very significant at $p<0.001$and ns represents non significant.

Figure 3.3.1.1: Effect of polyherbal formulation (EEPF) on body weight in control and diabetic rats.



3.3.1.2 Serum glucose:

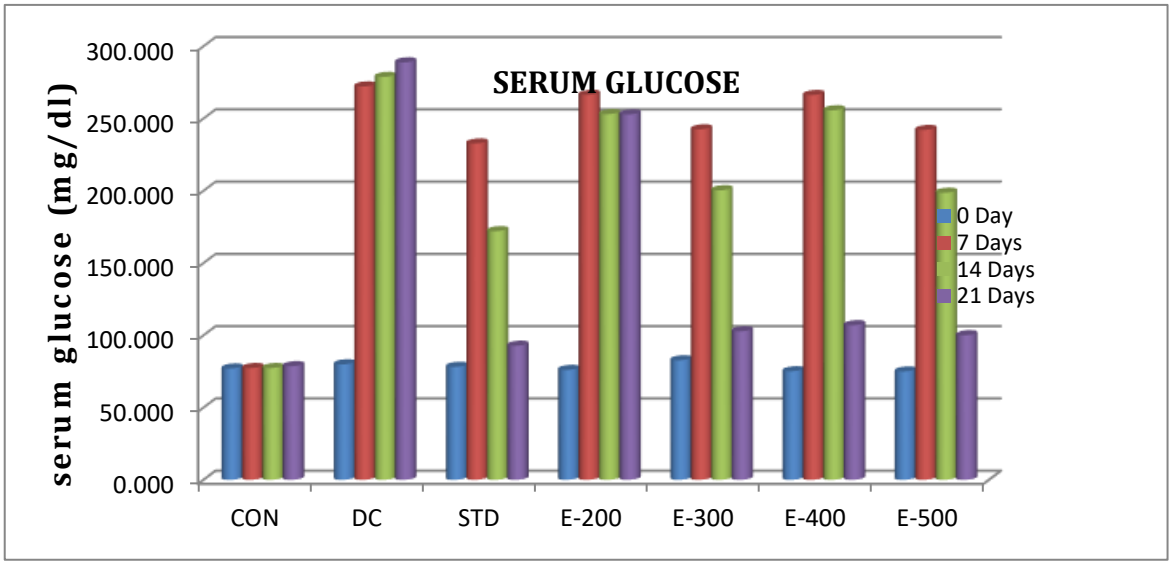
After 21 Days, diabetic rats exhibited significantly higher Serum glucose level (288.80±1.58) as compared to those of control rats (78.67 ± 2.78). Treatment with polyherbal formulation could significantly reduce the elevated glucose levels. EEPF-200 (252.80±2.26**), EEPF-300 (102.75±6.50***), EEPF-400 (106.95±4.28***) and EEPF-500 (100.00±4.07***). Glibenclamide treated rat's serum glucose level was (92.78±6.21***). However, the serum glucose levels in diabetic rats treated with polyherbal formulation were still found to be significantly higher than normal rats.

Table 3.3.1.2: Effect of polyherbal formulation (EEPF) on serum glucose in diabetic rats.

Groups	Treatment	Dose	Serum Glucose 0 days mg/dl	Serum Glucose7 days mg/dl	Serum Glucose 14 days mg/dl	Serum Glucose 21 days mg/dl
I	Control	10 ml/kg	77.03±4.38	77.32±4.50	77.32±4.14	78.67±2.78
II	Diabetic control stz	55 mg/kg	79.85±4.12	272.11±4.57	278.73±2.02	288.80±1.58
III	Glibenclamide	600 µg/kg	78.12±4.39	232.72±3.24***	172.04±4.28***	92.78±6.21***
IV	EEPF	200 mg/kg	76.05±3.50	266.35±4.59	253.01±4.45*	252.80±2.26**
V	EEPF	300 mg/kg	82.66±4.42	242.46±4.35**	200.31±5.80***	102.75±6.50***
VI	EEPF	400 mg/kg	75.09±3.18	266.21±3.10	255.64±5.51*	106.95±4.28***
VII	EEPF	500 mg/kg	74.95±5.58	242.01±4.06**	198.61±2.49***	100.00±4.07***

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, ***represents very significant at $p<0.001$and ns represents non significant.

Figure 3.3.1.2: Effect on serum glucose levels by chronic treatment with polyherbal formulation (EEPF) in control and diabetic rats.



3.3.1.3 Cholesterol Profile:

Diabetic rats exhibited significantly higher cholesterol (136.44 ± 3.60) as compared to those of control rats (92.42 ± 0.55). Treatment with polyherbal formulation could significantly reduce the elevated cholesterol levels. EEPF-200 (126.6 ± 0.24 *), EEPF-300 (109.11 ± 1.20***), EEPF-400 (114.66 ± 1.20**) and EEPF-500 (108.02 ± 1.24***). Glibenclamide treated rat's serum cholesterol level was (96.06 ± 1.31***).

Table 3.3.1.3: Effect of polyherbal formulation (EEPF) on serum cholesterol in diabetic rats.

Group	Treatment	Dose	Cholesterol levels (mg/dl) (Mean ± SEM)
I	Control	10 ml/kg	92.42 ± 0.55
II	Diabetic control stz	55 mg/kg	136.44 ± 3.60
III	Glibenclamide	600 µg/kg	96.06 ± 1.31***
IV	EEPF 200	200 mg/kg	126.6 ± 0.24 *
V	EEPF 300	300 mg/kg	109.11 ± 1.20***
VI	EEPF 400	400 mg/kg	114.66 ± 1.20**
VII	EEPF 500	500 mg/kg	108.02 ± 1.24***

Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, ***represents very significant at $p<0.001$and ns represents non significant.

3.3.1.4 Triglyceride Profile:

Diabetic rats exhibited significantly higher triglyceride (96.10 ± 1.15) as compared to those of control rats (50.35 ± 2.05). Treatment with polyherbal formulation could significantly reduce the elevated triglyceride levels. EEPF-200 (90.36 ± 035 *), EEPF-300 (78.40 ± 0.14***), EEPF-400 (79.80 ± 1.54***) and EEPF-500 (77.54 ± 1.11***). Glibenclamide treated rat's serum triglyceride level was (53.24 ± 1.52***).

Table 3.3.1.4: Effect of polyherbal formulation (EEPF) on serum triglyceride in diabetic rats.

Group	Treatment	Dose	Triglyceride (mg/dl) (Mean ± SEM)
I	Control	10 ml/kg	50.35 ± 2.05
II	Diabetic control stz	55 mg/kg	96.10 ± 1.15
III	Glibenclamide	600 µg/kg	53.24 ± 1.52***
IV	EEPF 200	200 mg/kg	90.36 ± 035 *
V	EEPF 300	300 mg/kg	78.40 ± 0.14***
VI	EEPF 400	400 mg/kg	79.80 ± 1.54***
VII	EEPF 500	500 mg/kg	77.54 ± 1.11***

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant.

3.3.1.5 HDL Profile:

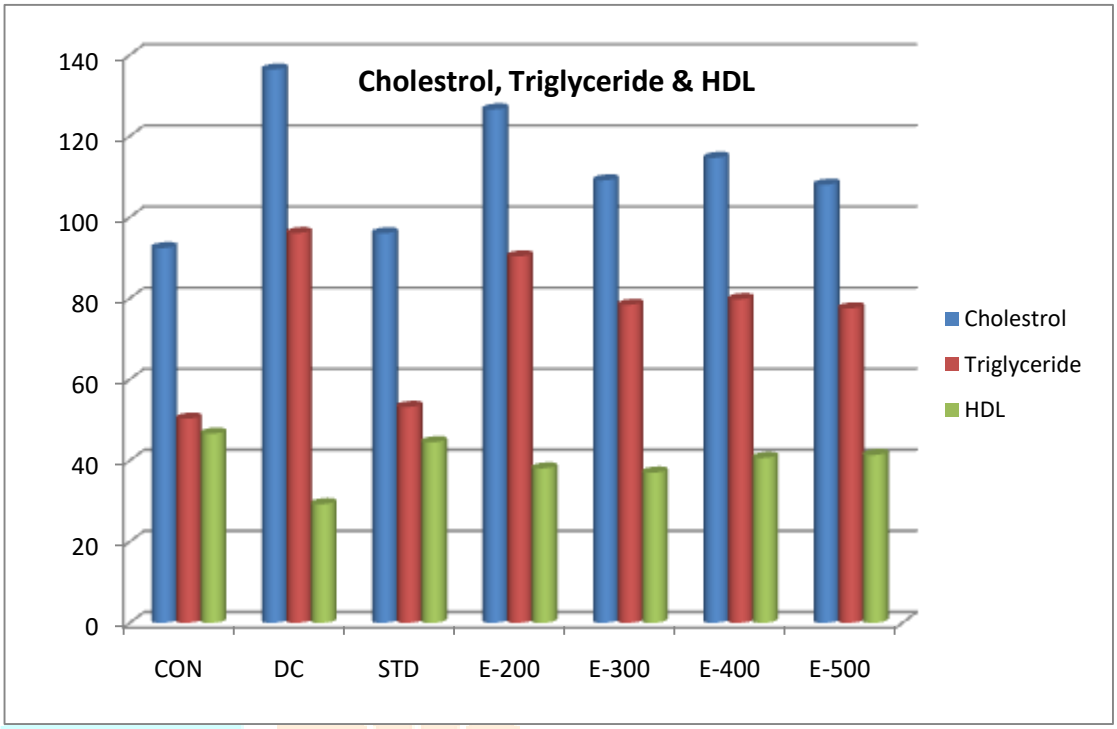
Diabetic rats exhibited significantly lower HDL (29.27 ±1.11) as compared to those of control rats (46.65 ± 2.35). Treatment with polyherbal formulation could significantly elevated the reduced HDL levels. EEPF-200 (38.09 ±1.39*), EEPF-300 (37.05 ±1.19*), EEPF-400 (40.66 ± 1.16***) and EEPF-500 (41.41 ± 1.13***). Glibenclamide treated rat's serum HDL level was (44.55 ± 2.24***).

Table 3.3.1.5: Effect of polyherbal formulation (EEPF) on serum HDL in diabetic rats.

Group	Treatment	Dose	HDL (mg/dl) (Mean ± SEM)
I	Control	10 ml/kg	46.65 ± 2.35
II	Diabetic control stz	55 mg/kg	29.27 ±1.11
III	Glibenclamide	600 µg/kg	44.55 ± 2.24***
IV	EEPF 200	200 mg/kg	38.09 ±1.39*
V	EEPF 300	300 mg/kg	37.05 ±1.19*
VI	EEPF 400	400 mg/kg	40.66 ± 1.16***
VII	EEPF 500	500 mg/kg	41.41 ± 1.13***

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant.

Figure 3.3.1.3-1.5: Effect on Cholesterol, triglyceride and HDL levels by chronic treatment with polyherbal formulation (EEPF) in control and diabetic rats



3.3.1.6 SGOT, SGPT & ALP PROFILE:

3.3.1.6.1 Serum glutamate oxaloacetate transaminase (SGOT):

Diabetic rats exhibited significantly higher SGOT (138.22 ± 2.18) as compared to those of control rats (72.98 ± 1.63). Treatment with polyherbal formulation could significantly reduce the elevated SGOT levels. EEPF-200 (128.15 ± 0.36**), EEPF-300 (127.19 ± 1.66**), EEPF-400 (101.22 ± 1.44***) and EEPF-500 (91.79 ± 2.04***) Glibenclamide treated rat's serum SGOT level was (86.59 ± 2.25***).

Table 3.3.1.6.1: Effect of polyherbal formulation (EEPF) on serum SGOT in diabetic rats.

Group	Treatment	Dose	SGOT levels (U/L) (Mean ±SEM)
I	Control	10 ml/kg	72.98 ± 1.63
II	Diabetic control stz	55 mg/kg	138.22 ± 2.18
III	Glibenclamide	600 µg/kg	86.59 ± 2.25***
IV	EEPF 200	200 mg/kg	128.15 ± 0.36**
V	EEPF 300	300 mg/kg	127.19 ± 1.66**
VI	EEPF 400	400 mg/kg	101.22 ± 1.44***
VII	EEPF 500	500 mg/kg	91.79 ± 2.04***

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant.

3.3.1.6.2 Serum glutamate pyruvate transaminase (SGPT) :

Diabetic rats exhibited significantly higher SGPT (96.20 ± 1.20) as compared to those of control rats (46.14 ± 2.71). Treatment with polyherbal formulation could significantly reduce the elevated SGPT levels. EEPF-200 (89.35 ± 2.37*), EEPF-300 (87.26 ± 2.69*), EEPF-400 (83.29 ± 2.55**) and EEPF-500 (81.98 ± 0.86**). Glibenclamide treated rat's serum SGPT level was (50.66 ± 0.56***).

Table 3.3.1.6.2: Effect of polyherbal formulation (EEPF) on serum SGPT in diabetic rats.

Group	Treatment	Dose	SGPT levels (U/L) (Mean ± SEM)
I	Control	10 ml/kg	46.14 ± 2.71
II	Diabetic control stz	55 mg/kg	96.20 ± 1.20
III	Glibenclamide	600 µg/kg	50.66 ± 0.56***
IV	EEPF 200	200 mg/kg	89.35 ± 2.37*
V	EEPF 300	300 mg/kg	87.26 ± 2.69*
VI	EEPF 400	400 mg/kg	83.29 ± 2.55**
VII	EEPF 500	500 mg/kg	81.98 ± 0.86**

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant.

3.3.1.6.3 Alkaline phosphate (ALP):

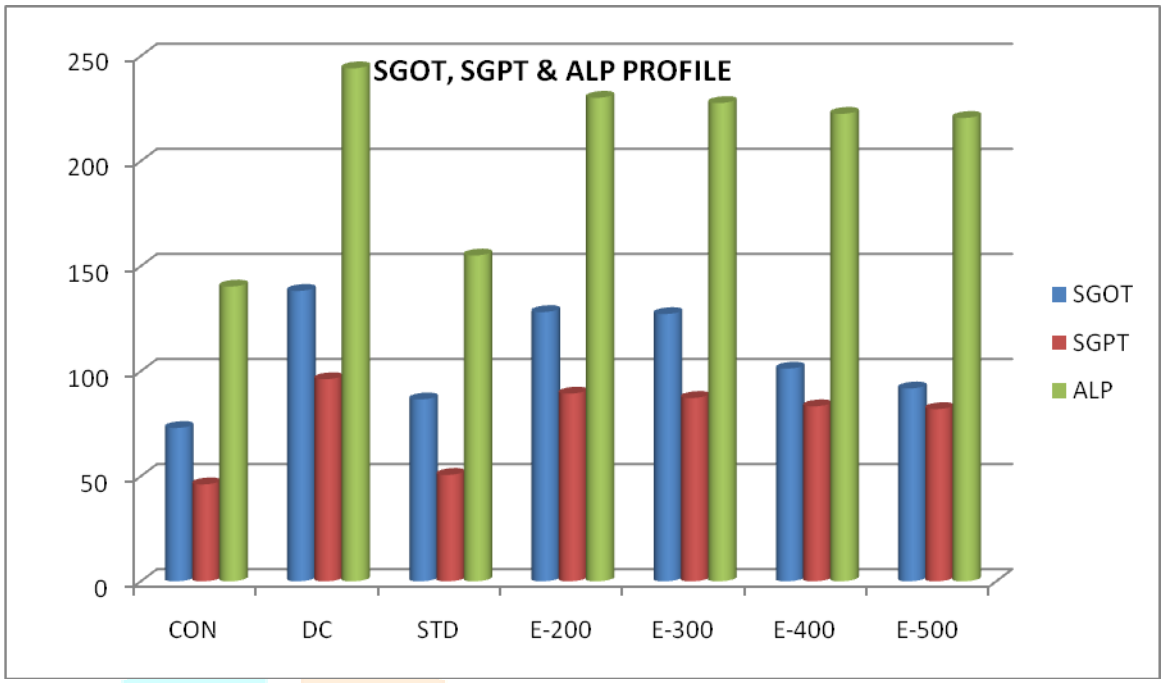
Diabetic rats exhibited significantly higher ALP (244.15 ± 1.69) as compared to those of control rats (140.29 ± 2.15). Treatment with polyherbal formulation could significantly reduce the elevated ALP levels. EEPF-200 (230.14 ± 1.91*), EEPF-300 (227.64 ± 2.95*), EEPF-400 (222.50 ± 4.29***) and EEPF-500 (220.53 ± 1.39***). Glibenclamide treated rat's serum ALP level was (155.07 ± 2.37***).

Table 3.3.1.6.3: Effect of polyherbal formulation (EEPF) on serum ALP in diabetic rats.

Group	Treatment	Dose	ALP levels (mg/dl) (Mean ± SEM)
I	Control	10 ml/kg	140.29 ± 2.15
II	Diabetic control stz	55 mg/kg	244.15 ± 1.69
III	Glibenclamide	600 µg/kg	155.07 ± 2.37***
IV	EEPF 200	200 mg/kg	230.14 ± 1.91*
V	EEPF 300	300 mg/kg	227.64 ± 2.95*
VI	EEPF 400	400 mg/kg	222.50 ± 4.29***
VII	EEPF 500	500 mg/kg	220.53 ± 1.39***

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant.

Figure 3.3.1.6: Effect on SGOT, SGPT & ALP levels by chronic treatment with polyherbal formulation (EEPF) in control and diabetic rats.



3.3.1.7 Total protein content:

Diabetic rats exhibited significantly lower total protein (2.57 ± 0.21) as compared to those of control rats (6.48 ± 0.37). Treatment with polyherbal formulations could significantly elevated the reduced total protein levels. EEPF-200 (2.36 ± 0.32), EEPF-300 (2.58 ± 0.50), EEPF-400 (4.77 ± 0.43**) and EEPF-500 (5.13 ± 0.40*). Glibenclamide treated rat's serum total protein level was (5.53 ± 0.17***).

Table 3.3.1.7: Effect of polyherbal formulation (EEPF) on serum total protein in diabetic rats.

Group	Treatment	Dose	Total protein(mg/dl) (Mean ± SEM)
I	Control	10 ml/kg	6.48 ± 0.37
II	Diabetic control stz	55 mg/kg	2.57 ± 0.21
III	Glibenclamide	600 µg/kg	5.53 ± 0.17**
IV	EEPF 200	200 mg/kg	2.36 ± 0.32
V	EEPF 300	300 mg/kg	2.58 ± 0.50
VI	EEPF 400	400 mg/kg	4.77 ± 0.43**
VII	EEPF 500	500 mg/kg	5.13 ± 0.40**

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant.

3.3.1.8 Creatinine Profile:

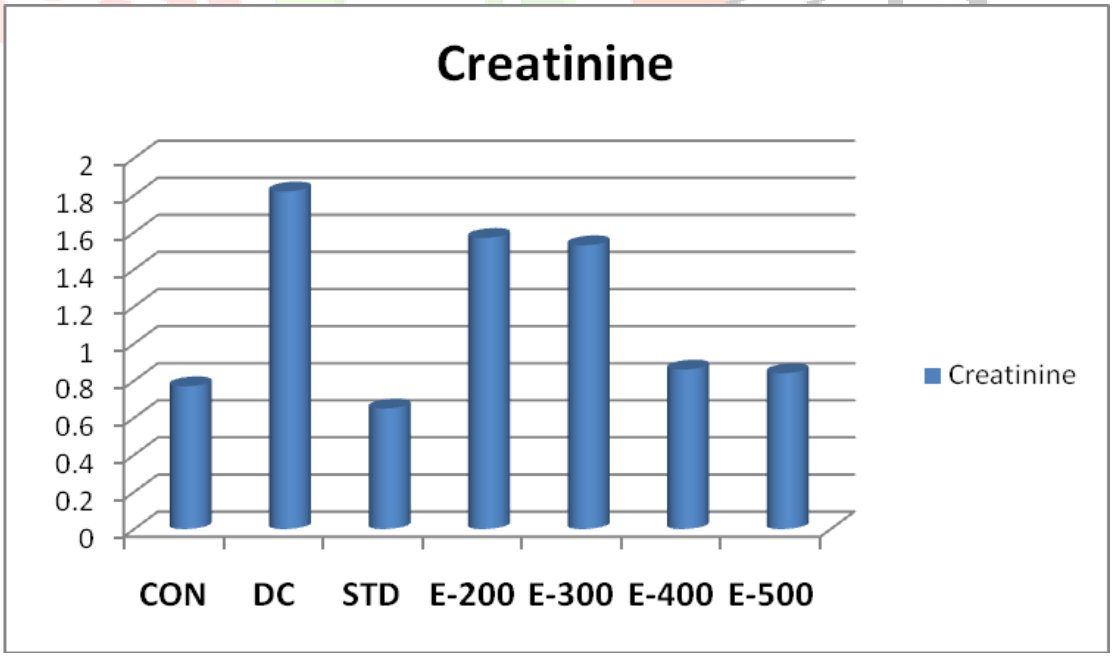
Diabetic rats exhibited significantly higher Creatinine (1.82 ± 0.47) as compared to those of control rats (0.77 ± 0.02). Treatment with polyherbal formulation could significantly reduce the elevated Creatinine levels. EEPF-200 (1.57 ± 0.21), EEPF-300 (1.53 ± 0.30), EEPF-400 (0.86 ± 0.04*) and EEPF-500 (0.84 ± 0.04*).Glibenclamide treated rat's serum creatinine level was (0.65 ± 0.05**).

Table 3.3.1.8: Effect of polyherbal formulation (EEPF) on serum creatinine in diabetic rats.

Group	Treatment	Dose	Creatinine (mg/dl) (Mean ± SEM)
I	Control	10 ml/kg	0.77 ± 0.02
II	Diabetic control stz	55 mg/kg	1.82 ± 0.47
III	Glibenclamide	600 µg/kg	0.65 ± 0.05**
IV	EEPF 200	200 mg/kg	1.57 ± 0.21
V	EEPF 300	300 mg/kg	1.53 ± 0.30
VI	EEPF 400	400 mg/kg	0.86 ± 0.04*
VII	EEPF 500	500 mg/kg	0.84 ± 0.04*

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant.

Figure 3.3.1.8: Histograph of Effect of polyherbal formulation (EEPF) on serum creatinine in diabetic rats.



3.3.1.9 Super Oxide dismutase (SOD) Profile:

Diabetic rats exhibited significantly lower SOD (2.053 ± 0.12) as compared to those of control rats (4.81 ± .011). Treatment with polyherbal formulations could significantly elevated the reduce SOD levels. EEPF-200 (2.91 ± 0.015**), EEPF-300 (2.90 ± 0.032**), EEPF-400 (3.10±0.020***) and EEPF-500 (3.15 ± 0.012***). Glibenclamide treated rat's SOD level was (4.15 ± 0.22***).

Table 3.3.1.9: Effect of polyherbal formulation (EEPF) on SOD in Diabetic rats.

Group	Treatment	Dose	SOD
I	Control	10 ml/kg	4.81 ± .011
II	Diabetic control stz	55 mg/kg	2.053 ± 0.12
III	Glibenclamide	600 µg/kg	4.15 ± 0.22***
IV	EEPF 200	200 mg/kg	2.91 ± 0.015**
V	EEPF 300	300 mg/kg	2.90 ± 0.032**
VI	EEPF 400	400 mg/kg	3.10 ± 0.020 ***
VII	EEPF 500	500 mg/kg	3.15 ± 0.012***

Unit: Units/ mg Protein

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant.

3.3.1.10 Catalase Profile:

Diabetic rats exhibited significantly lower Catalase (5.51 ± 0.24) as compared to those of control rats (12.63±0.20). Treatment with Polyherbal formulation could significantly elevated the reduce Catalase levels. EEPF-200 (7.46 ±0.25*), EEPF-300 (7.15 ± 0.49*), EEPF-400 (7.88 ± 0.18**) and EEPF-500 (7.85 ± 0.33**). Glibenclamide treated rat's catalase level was (12.12 ± 0.41***).

Table 3.3.1.10: Effect of polyherbal formulation (EEPF) on catalase in diabetic rats.

Group	Treatment	Dose	Catalase
I	Control	10 ml/kg	12.63 ± 0.20
II	Diabetic control stz	55 mg/kg	5.51 ± 0.24
III	Glibenclamide	600 µg/kg	12.12 ± 0.41***
IV	EEPF 200	200 mg/kg	7.46 ±0.25*
V	EEPF 300	300 mg/kg	7.15 ± 0.49*
VI	EEPF 400	400 mg/kg	7.88 ± 0.18**
VII	EEPF 500	500 mg/kg	7.85 ± 0.33**

Unit: µmole of H_2O_2/ Sec/mg protein/ml

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant.

3.3.1.11 Glutathione peroxidase (GPx) :

Diabetic rats exhibited significantly lower GPx (8.02 ± 0.12) as compared to those of control rats (18.02 ± 0.11). Treatment with Polyherbal formulation could significantly elevated the reduce GPx levels. EEPF-200 (9.99 ± 0.22*), EEPF-300 (10.06 ± 0.44*), EEPF-400 (10.52 ±0.28**) and EEPF-500 (10.75 ± 0.47**). Glibenclamide treated rat's GPx level was (16.04 ± 0.28***).

Table 3.3.1.11: Effect of polyherbal formulation (EEPF) on GPx in diabetic rats.

Group	Treatment	Dose	GPx
I	Control	10 ml/kg	18.02 ± 0.11
II	Diabetic control stz	55 mg/kg	8.02 ± 0.12
III	Glibenclamide	600 µg/kg	16.04 ± 0.28***
IV	EEPF 200	200 mg/kg	9.99 ± 0.26*
V	EEPF 300	300 mg/kg	10.06 ± 0.44*
VI	EEPF 400	400 mg/kg	10.52 ±0.28**
VII	EEPF 500	500 mg/kg	10.75 ± 0.47**

Unit: µ mole of the oxidized GPx / min × mg protein

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant.

3.3.1.12 Lipid Peroxidation (LPO):

Diabetic rats exhibited significantly higher LPO (57.42 ± 1.15) as compared to those of control rats (32.21 ± 0.90). Treatment with Polyherbal formulations could significantly reduce the elevated LPO levels. EEPF-200 (52.29 ± 0.68*), EEPF-300 (52.58 ± 0.63*), EEPF-400 (50.47 ± 1.12 **) and EEPF-500 (51.06 ± 0.51**). Glibenclamide treated rat's LPO level was (40.37 ± 0.25***).

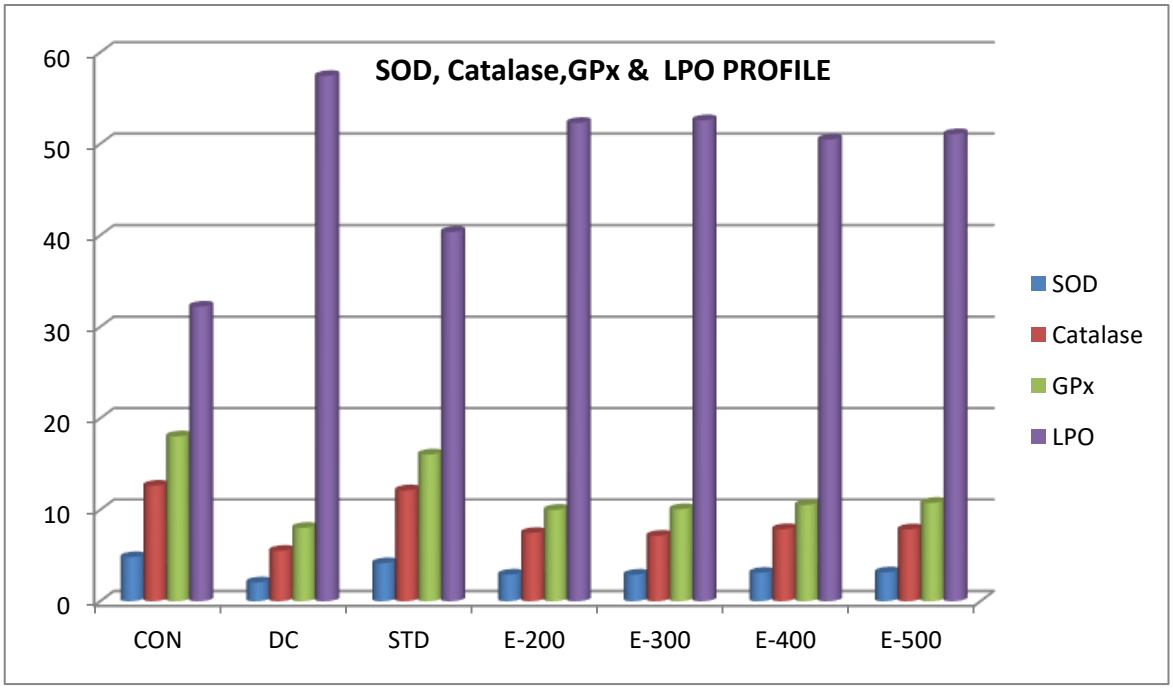
Table 3.3.1.12: Effect of polyherbal formulation (EEPF) on LPO in diabetic rats.

Group	Treatment	Dose	LPO
I	Control	10 ml/kg	32.21 ± 0.90
II	Diabetic control stz	55 mg/kg	57.42 ± 1.15
III	Glibenclamide	600 µg/kg	40.37 ± 0.25***
IV	EEPF 200	200 mg/kg	52.29 ± 0.68*
V	EEPF 300	300 mg/kg	52.58 ± 0.63*
VI	EEPF 400	400 mg/kg	50.47 ± 1.12 **
VII	EEPF 500	500 mg/kg	51.06 ± 0.51**

Unit: nmol MDA / min × mg protein

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey-kramer's test. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$and ns represents non significant**.**

Figure 3.3.1.9-12: Effect on SOD, CATALASE, GPx & LPO levels by chronic treatment with polyherbal formulation (EEPF) in control and diabetic rats.



3.4 Histopathology Reports

3.4.1 Normal Control Microscopy:

Section studied shows pancreatic lobules separated by connective tissue septa. The pancreatic lobules consist largely of the exocrine acini and their intralobular ducts. Most of the lobules show small, round, light-staining islets of langerhans. The center of islet cells consist of aggregates of small Beta-cells (70%, Fig.3.4.1/1, arrow) having basophilic granules, while the periphery comprises of large Alpha-cells (25% Fig.3.4.1/2, double-arrow) having eosinophilic granules. Intervening these cells are seen thin walled capillaries.

3.4.2 Diabetic Control (Streptozotocin) Microscopy:

Section studied shows pancreatic lobules separated by connective tissue septa. Some of the lobules show small, round, light-staining islets of langerhans (Fig.3.4.2/1). The center of islet cells consist of quantitative decrease in Beta-cells (40%, Fig.3.4.2/2) having basophilic granules, while the periphery comprises of large Alpha-cells (55% Fig.3.4.2) having eosinophilic granules. Also seen are scattered lymphocytes within the islet cells.

Figure 3.4.1: Photographs of Histopathological tissues of Normal Control

NORMAL CONTROL Fig 1 **NORMAL CONTROL Fig 2**

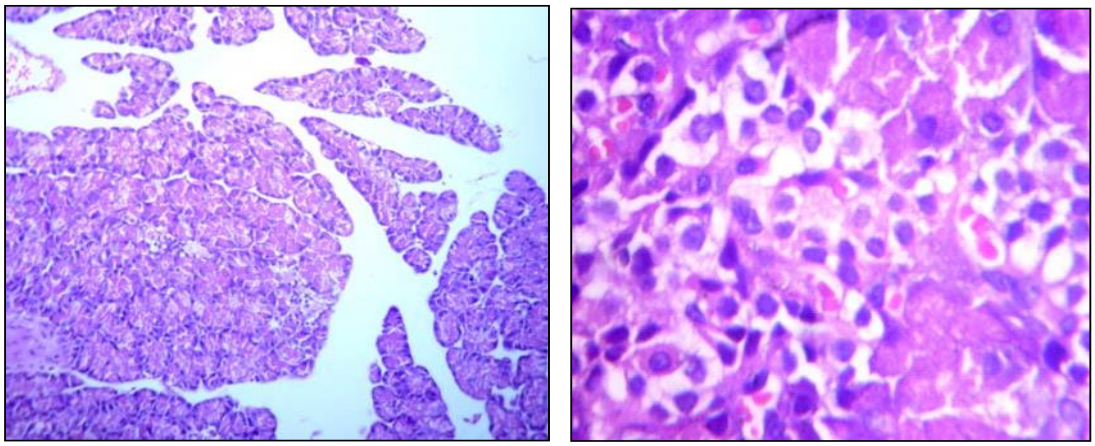
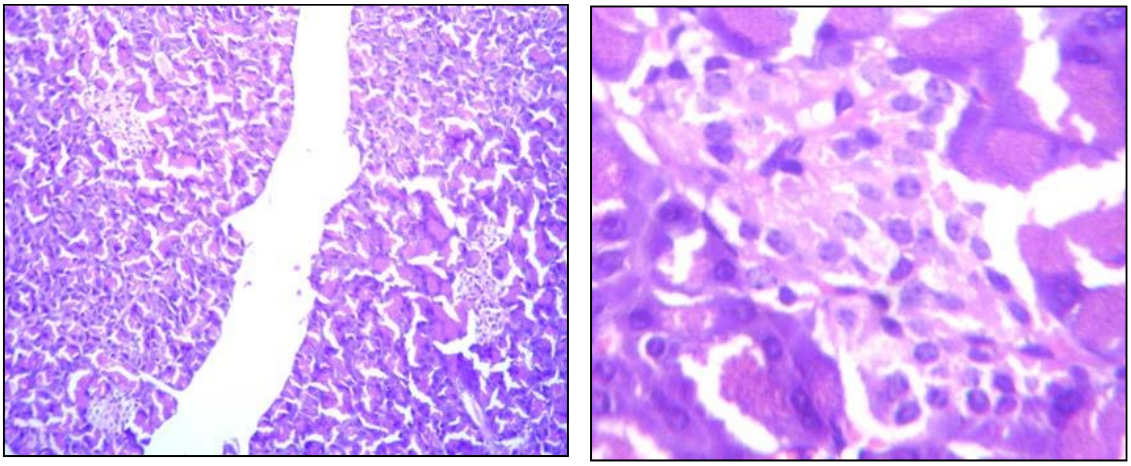


Figure 3.4.2: Photographs of Histopathological tissues of Diabetic Control DIABETIC CONTROL Figure.1 DIABETIC CONTROL Figure.2

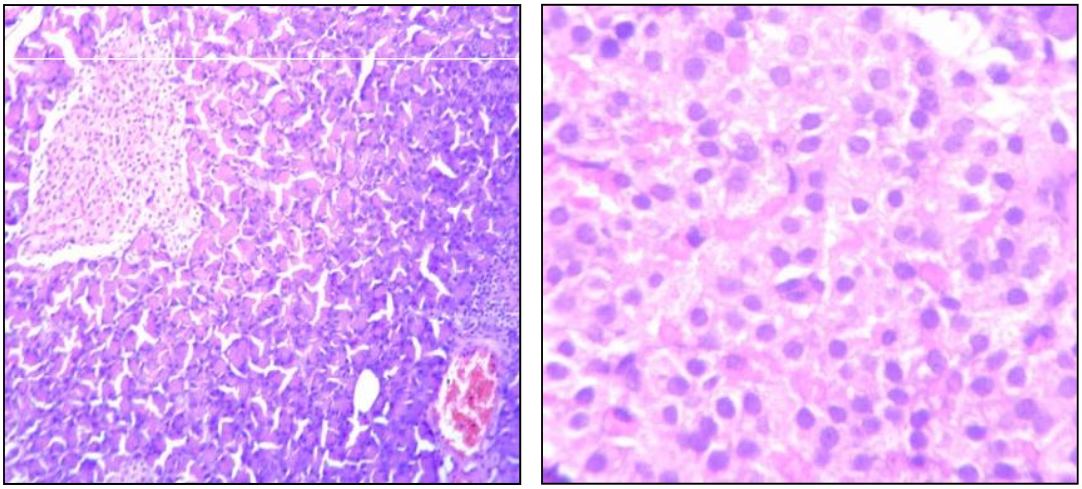


3.4.3 Standard (Glibenclamide) Microscopy:

Section studied shows pancreatic lobules separated by connective tissue septa. Most of the lobules show large areas of light-staining islets of langerhans (Fig.3.4.3/1). The center of islet cells consist of mild quantitative increase in Beta-cells (75%, Fig.3.4.3/2) having basophilic granules, while the periphery comprises of Alpha-cells having eosinophilic granules. Also seen are congested vascular spaces (Fig.3.4.3) amidst these cells.

Figure 3.4.3: Photographs of Histopathological tissues of Standard(Glibenclamide)

STANDARD (Glibenclamide) Fig 1 **STANDARD(Glibenclamide) Fig 2**



3.4.4.1 EEPF 200mg Microscopy:

Section studied shows pancreatic lobules separated by connective tissue septa. Most of the lobules show areas of light-staining islets of langerhans (Fig.3.4.4.1.1). The center of islet cells consist of mild quantitative increase in Beta-cells (80%, Fig.3.4.4.1.2) having basophilic granules, while the periphery comprises of Alpha-cells having eosinophilic granules.

3.4.4.2 EEPF 300mg Microscopy:

Section studied shows pancreatic lobules separated by thin connective tissue septa. Most of the lobules show large areas of light-staining islets of langerhans (Fig. 3.4.4.1.3). The center of islet cells consist of moderate quantitative increase in Beta-cells (85%, Fig. 3.4.4.1.4) having basophilic granules, while the periphery comprises of Alpha-cells (10%) having eosinophilic granules.

3.4.4.3 EEPF 400mg Microscopy:

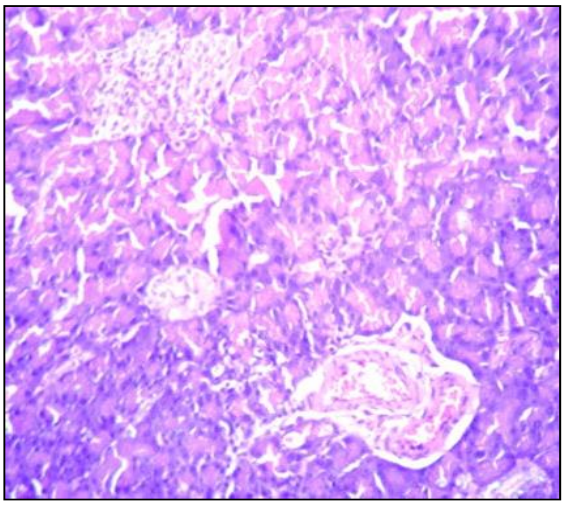
Section studied shows pancreatic lobules separated by thin fibrovascular septa. Most of the lobules show small areas of light-staining islets of langerhans (Fig. 3.4.4.1.5). The center of islet cells consist of quantitative decrease in Beta-cells (30%, Fig. 3.4.4.1.6) having basophilic granules, while the periphery comprises of Alpha-cells having eosinophilic granules (30%, Fig. 3.4.4.1.6). Also seen few scattered lymphocytic infiltration within the islet cells.

3.4.4.4 EEPF 500mg Microscopy:

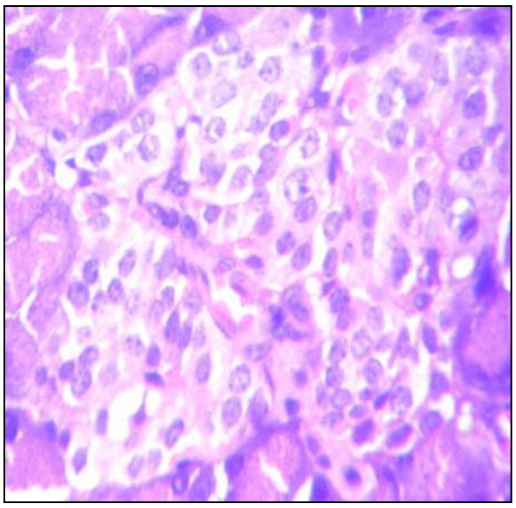
Section studied shows pancreatic lobules separated by thin connective tissue septa. Most of the lobules show very small areas of light-staining islets of langerhans (Fig. 3.4.4.1.7). The center of islet cells consist of quantitative decrease in Beta-cells (50%, Fig. 3.4.4.1.8) having basophilic granules, while the periphery comprises of Alpha-cells (70%, Fig. 3.4.4.1.8) having eosinophilic granules.

Figure 3.4.4.1-8: Photographs of Histopathological tissues of EEPF Treated 200mg,300mg,400mg & 500mg

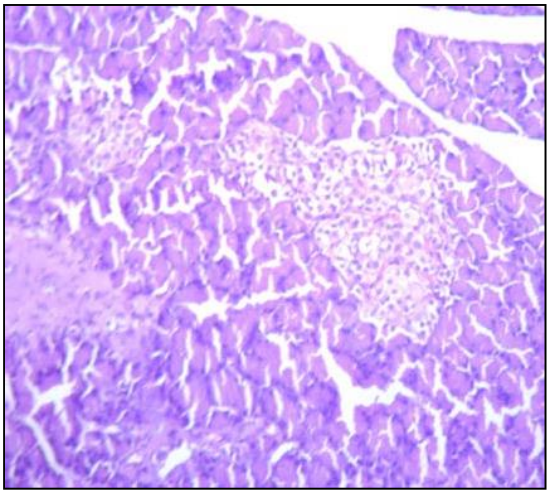
3.4.4.1.1.EEPF Treated 200mg



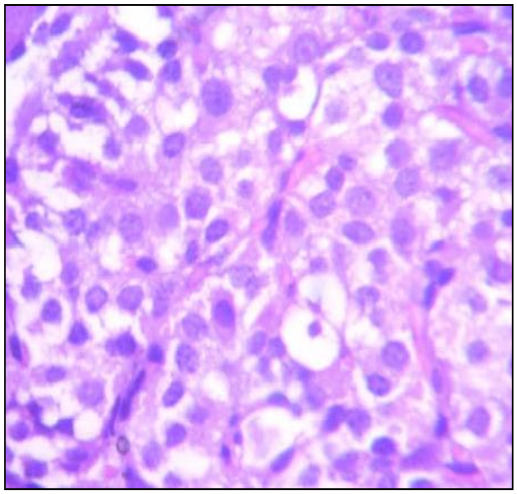
3.4.4.1.2.EEPF Treated 200mg



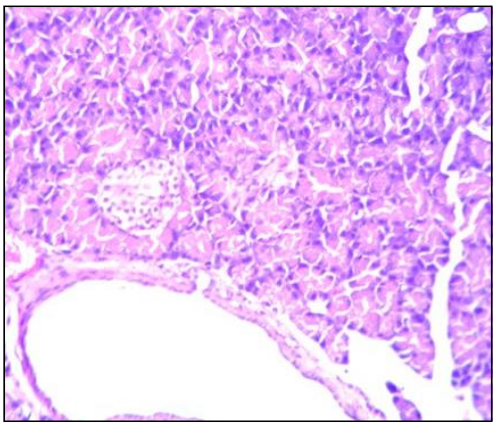
3.4.4.1.3.EEPF Treated 300mg



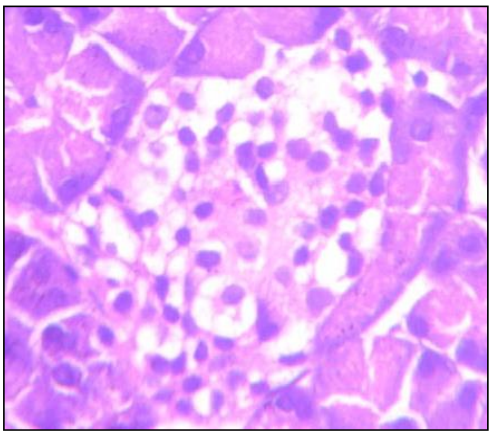
3.4.4.1.4.EEPF Treated 300mg



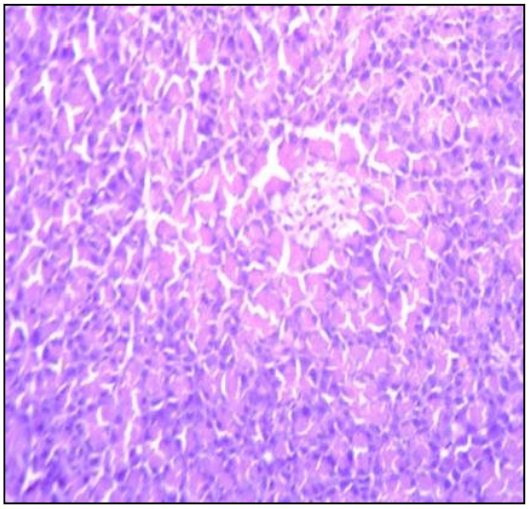
3.4.4.1.5.EEPF Treated 400mg

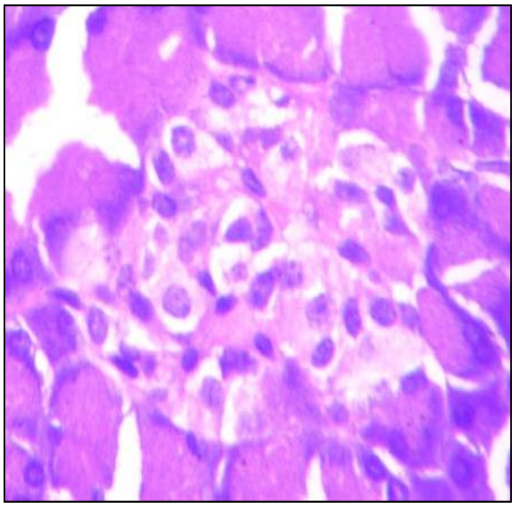


3.4.4.1.6. EEPF Treated 400mg



3.4.4.1.7.EEPF Treated 500mg **3.4.4.1.8. EEPF Treated 500mg**





4 Conclusion

The ethanolic extract of prepared poly herbal formulation containing seven medicinal plants of *Gymnema sylvestre*, *Trigonella foenum-graceum*, *Tinospora cardifolia* and *Azadirachata indica*, *Syzygium jambolana* and *Nardostachys jatamansi* were selected on the basis of their literature claim for hypoglycemic/antidiabetic & antioxidant effects. Present research work deals with polyherbal formulation development and screening of phytochemicals from ethanolic herbal extracts of *Gymnema sylvestre*, *Trigonella foenum-graceum*, *Tinospora cardifolia* and *Azadirachata indica*, *Syzygium jambolana* and *Nardostachys jatamansi* were prepared.

From this, polyherbal formulation exhibhited good antidiabetic & antioxidant activity. The maximum anti diabetic activity was reported which was found to be significant in at 400 & 500mg/Kg b.w. dose. Also, the biochemical examination of blood and histopathology studies also carried out. Hence, these investigations provide strong support for the selected medicinal plants contained in the polyherbal formulation for this research work and which also which ascertain its folk claims.

The present research work may be helpful in development of efficacious and potent polyherbal formulations in diabetes mellitus treatment. From the present work it is concluded that the polyherbal formulation was successfully prepared and found stable at 40°C. The formulation prepared is unique in it containing natural anti-oxidants for the oxidizable part of extracts. Here the present researcher creates a thrust for the future researchers to standardize the selected polyherbal formulation for better optimization.

The polyherbal formulation containing multiple ethanolic herbal extracts from various herbs which are routinely consumed by the human beings in daily life.Moreover, further studies are required for isolation, characterization and purification of active component(s) from most active extracts which might have a good complementary and/or independent regimen for the treatment of diabetes.

To conclude, the polyherbal formulation containing selected plant extracts shows a promising antidiabetic activity in dose dependent manner and the prepared polyherbal formulation EEPF proven to have antidiabetic effect and it may be used as an alternative remedy for the treatment of diabetes mellitus.

5 Bibliography

1. F. Builders P. Introductory Chapter: Introduction to Herbal Medicine. Jan 30th 2019.
2. W. Arika, C. M. Kibiti, J. M. Njagi, and M. P. Ngugi, "In vitro antioxidant properties of dichloromethanolic leaf extract of Gnidia glauca (Fresen) as a promising antiobesity drug,"*Journal of Evidence-Based Integrative Medicine*, vol. 24, 2019.
3. A. H. Bhat, K. B. Dar, S. Anees et al., "Oxidative stress,mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight," *Biomedicine & Pharmacotherapy*,vol. 74, pp.101–110, 2015.
4. G. A. Moriasi, A. M. Ireri, and M. P. Ngugi, "In vivo cognitiveenhancing, ex vivo malondialdehyde-lowering activities and phytochemical profiles of aqueous and methanolic stem bark extracts of Piliostigma thonningii (schum.)," *International Journal of Alzheimer's Disease*, vol. 2020, Article ID 1367075,15 pages, 2020.
5. S. Vertuani, A. Angusti, and S. Manfredini, "&e antioxidants and pro-antioxidants network: an overview," *Current Pharmaceutical Design*, vol. 10, no. 14, pp. 1677–1694, 2004.
6. A. Ndhlala, M. Moyo, and J. Van Staden, "Natural antioxidants:fascinating or mythical biomolecules?" *Molecules*,vol. 15, no. 10, pp. 6905–6930, 2010.
7. S. K. Mwihia, "In vitro antibacterial and antioxidant activities of methanolic and dichloromethanolic seed extracts of Kenyan Annona squamosa Linn," Doctoral dissertation,Kenyatta University, Nairobi, Kenya, 2017.
8. M. R. Goyal and H. A. R. Suleria, Eds., *Human Health Benefits of Plant Bioactive Compounds: Potentials and Prospects*, CRC Press, Boca Raton, FL, USA, 2019.
9. C. Ojiewo, A. Tenkouano, J. D. A. Hughes, and J. D. H. Keatinge, "Diversifying diets: using indigenous vegetables to improve profitability, nutrition and health in Africa," *Diversifying Food and Diets: Using Agricultural Biodiversity to Improve Nutrition and Health*, pp. 291–302, Routledge, Abingdon, UK, 2013.
10. C. B. Rajashekar, E. E. Carey, X. Zhao, and M. M. Oh, "Healthpromoting phytochemicals in fruits and vegetables: impact of abiotic stresses and crop production practices," *Functional Plant Science and Biotechnology*, vol. 3, no. 1, pp. 30–38, 2009.
11. A. N. Panche, A. D. Diwan, and S. R. Chandra, "Flavonoids: an overview," *Journal of Nutritional Science*, vol. 5, 2016.
12. Paul Cos, J. Arnold and Vlietinck, J. Ethnopharmacol., 4, 290-302, 2006.
13. A. Prusti and S. R. Mishra, Botanical Leaflets, 12, 227-230, 2008.
14. Wallia TE. Text book of Pharmacognosy. 5th ed. New delhi: CBS Publishers and Distributors; 1985.
15. Khandelwal KR, Kokate CK, Pawar AP and Gokhle SB. Practical Pharmacognosy. 1st ed. Niraliprakashan; 1995.
16. OECD Guidelines for testing of chemical: OECD/OCDE 425:30 17th Dec. Acute Oral Toxicity – Up –and-Down Procedure; 2001:1-26.

17. OECD 2001- Guideline on acute oral toxicity, Environmental health and safety monograph series on testing and adjustment, no.425.
18. M.C.Tchamadeu, P.D.D.Dzeufiet, C.C.Kouambou Nouga, A.G.B.AZEBAZE, J.Allard, Jp.Girolami et al. Hypoglycaemic effect of *Mammea africana* (Guttiferae) in diabetic rats. J Ethnopharmacol 2009; 127: 368-372.
19. Pari L, Uma Maheshwari J. Antihyperglycemic activity of *Musa Sapentium* flower: Effect on lipid peroxidationin alloxan diabetic rats. Phytother Res 2000; 14: 1-3.
20. Trinder P. Determination of blood glucose using an oxidase–perioxidase system with a noncarcinogenic chromogen. J. Clinical Pathology 1969; 22: 158-161.
21. Trinder P. Ann.Clin.Biochem 1969; 6:24.
22. Allain cc te al. clin Chem 1974; 20:74.
23. Flegg HM.Ann clin Biochem 1972; 10:79.
24. Grillo F et al. Clin Chem 1981; 27:375.
25. Demacker PNM. Clin Chem 1980; 26:1775.
26. Bowers GN and McCommb RB. Clin Chem 1972;18:97.
27. Bowers LD et al. Clin Chem 1980;26:551.
28. Bowers LD et al. Clin Chem 1980;26:655.
29. Bowers LD et al. Clin Chem 1980;26:655.
30. IFCC methods for the measurement of catalytic concentration of enzymes. J. Clin. Chem.Clin Biochem 1986; 24:481-497.
31. Gornall AG *et al.* Biol. Chem 1949; 177:751.
32. Ohkawa, H., Ohishi N., Yagi, K., Assay for lipid peroxidation in animal tissueby thiobarbituric acid reaction. Anal Biochem 1979; 95; 51-58.
33. Rotruck, J.T, Pope, A.L., Ganther, H.L., and Swanson, A.B., Selenium: biochemical role as a component of glutathione peroxidase. Science 1973; 179,588-590.
34. Marklund, A., Marklund G., Involvement of superoxide anion radical in theautoxidation of pyrogallol and a convenient assay for superoxide dismutase.Eur. J. Biochem 1974; 47, 469-474.
35. Sinha AK. Colorimetric assay of catalase. Anal. Biochem 1972; 47:389-394.
36. Pomory CM. Color development time of the Lowry protein assay. AnalBiochem 2008; 378:216-7.