World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org

EVALUATION HAEMATOLOGICAL PARAMETERS OF POLYHERBAL PREPARATION ON STREPTOZOTOCIN INDUCED DIABETIC RATS.

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Article Received on 02/07/2015 Article Revised on 25/07/2015 Article Accepted on 17/08/2015

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ABSTRACT

Objective: The current sudy was designed to evaluate the anti-diabetic and haematological parameters of combined poly herbal extract preparaion on streptozotocin (60mg/kg bw) induced diabetic rats. *Asistasia gangetica* leaf extract (AGLE-50mg/kg), *Ficus racemosa* leaf extract (FRLE-75mg/kg) and *Morus indica* leaf extract (MILE-75mg/kg) were combinely (poly herbal preparation-PHP) administered to diabetic and non diabetic rats. In this study Glibenclamide (5mg/kg) is used as standard drug.[2,3,5] The physiological animal body weight (BW), haematological parameters like Fasting blood glucose (FBG), Oral Glucose Tollarance Test(OGTT), RBC counts (RBCc), WBC counts (WBCc), Haemoglobin level (Hb), Glycocylated Haemoglobin (HbA_1C), bleeding time (Bt-duke method), cloting time (Ct-capillary glass method), were studied were studied.[13]

KEYWORDS: Streptozotocine, anti-diabetic, *Asistasia gangetica*, *Ficus racemosa*, *Morus indica*, Haematological.

1. INTRODUCTION

The word “diabetes = siphon or drain off” is Greek word. “Mellitus = Sweet” is a Latin word.

1.1 *Diabetes mellitus* (DM) is an endocrine disorder characterized by hyperglycaemia, is associated with disturbances of carbohydrate, fat and protein metabolism resulting from

defects in insulin secretion and insulin action or both.[1] The majority of the cases of diabetes fall in to two broad etio-pathogenic categories i.e., ***type-1*** (IDDM) diabetes due to deficiency of insulin secretion and β-cell destruction. ***Type-2*** (NIDDM) diabetes due to resistance to insulin action and an inadequate compensatory insulin-secretary response[1] Also ***Gestational diabetes*** and ***others*** like i) Genetic defects of β-cell function, ii). Genetic defects in insulin action, iii). Exocrine pancreas Diseases (Pancreatitis, Neoplasia and Cystic fibrosis), iv). Endocrinopathies (Acromegaly, Cushings syndrome and Pheochromacytoma), v). Drug/chemical induced (Nicotinic acid, Glucocorticoids and Thiazides), vi). Infections (Congenital rubella and Cytomegalovirus), vii). Other genetic syndromes (Downs syndrome, Klinefelters syndrome and Turner syndrome).[1]

1.2 Epidemiology

Globally 285 million people had diabetes in 2010 with type 2 making up about 90% of the cases. Its incidence is expected to be almost doubled by 2030,[5] due to urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet.[5] In India there are more diabetics than any other country in the world, according to the International Diabetes Foundation, although more recent data suggest that China has even more. The disease affects more than 50 million Indians i.e., 7.1% of the nation's adults and kills about 1 million Indians per year. The average age on onset is 42.5 years. The high incidence is attributed to a combination of genetic susceptibility with adoption of a high-calorie, low-activity lifestyle by India's growing middle class.

1.3 Therapeutic role of phytomedicine in the management of diabetes mellitus

Depending on the nature of disease, insulin and certain synthetic drugs like Sulphonylureas, Biguanidines and Acarbose are widely used for diabetic treatment. In recent years, evidence of cases of “insulin resistance” and the occurrence of side effects from prolonged administration of conventional drugs have triggered the search for safe and effective alternatives. Several isolated phyto-chemicals have been examined for anti-diabetic activity as alternative treatment strategies for diabetes even certain resistant cases of diabetes that do not respond well to conventional drugs.

Herbal remedy the ancient healing system from India has steadily increased in popularity in the western world in recent years. Treatment with specific herbs and minerals to cure various diseases are familiar now a days. The botanicals in the Ayurvedic Meteria Medica have been proven to be safe and effective, through several hundred to thousand years of use. The

positive role of traditional medicinal plant in the prevention or control of some metabolic disorders like diabetes, cardiac disease and certain types of cancer. The great advantages of these medicinal plants are easily available and have no proven side effects. Oral hypoglycaemic drugs have been frequently used for controlling non-insulin dependent diabetes mellitus but synthetic medicines have side effects.

The activities of different key enzymes (hexokinase, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and lactase dehydrogenase) can be maintained by several medicines. For eg; to manage post-prandial hyperglycaemia(PPHG) at digestive level, modern medicine has α-glucosidase inhibitors(Acarbose, Miglitol, voglibose), to tackle insulin insufficiency and insulinotropic action at β-cells of pancreas, sulphonylureas (glibenclamide, glypizide) to enhance glucose uptake, Biguanids (metformin) and to manage the problem of insulin resistance, insulin sensitizers(glitazones) are developed.[13]

Medicinal plants and herbs are of great importance to the health of individuals and communities. Despite the existence of herbal medicines over many centuries, only relatively small number of plant species has been studied for their application. However, in the recent past, an increasing research evidence is getting accumulated, which clearly indicate the positive role of traditional medicinal plants in the prevention or control of some metabolic disorders like diabetes. The hypoglycaemic and hypolipidemic effect of various traditional medicinal plants like fenugreek, Jambu and bitter guard are reported but, there is limited information on the nutritional and anti-nutritional factors of herbal medicinal plants which lowers the blood glucose and lipid profile.

2. MATERIALS AND METHODS

2.1 Drugs and chemicals

Streptozotocin purchased from M/S.Otto chemicals, Mumbai. Glibenclamide was obtained from Cadila healthcare limited., Ahmadabad. Hb and HbA_1C KIT kit from Agappe diagnosis, Kerala. **SD** *CHECK (*blood glucose test strip-GOLD) from SD biosensor, Germany. Standard rat pelleted diet (Hindustan Lever Limited, Mumbai, India). All other chemicals used in the study were of analytical grade procure from respect manufacturers.

2.2 plant materials

The fresh leaves of *Asystasia gangetica* had been collected from Marudhamalai hills of Coimbatore district, during the month of may and June 2012 (senthilkumar,et al.,2006). The

plant was identified and authenticated by Dr.G.V.S. Moorthy, joint director, Botanical survey of India, Tamilnadu agricultural university(TNAU), Coimbatore, India and the voucher specimen has been given the code BSI/SC/5/23/07-08/Tech-290 dated 05.06.2007. *Ficus recemosa* (19-08-2010) and *Morus indica* (03-04-2009) were collected from Yogimallavaram, Tirupathi-chattoor district(A.P) and Karvettinagaram, chittoor district(AP) respectively and were authenticated by Dr.Madavachetty.,Assistant professor, Department of botany, Sri Venkateswra University, Tirupathi(AP).

2.3 preparation of the extract

The fresh leaves of the plants were collected, air dried under shade at room temperature, powdered mechanically and stored in air tight container for experimental purposes separately. Maceration technique was followed for extraction of using 70% ethanol as solvent.[9] About 40gm of powder was mixed with 150ml of solvent and kept on mechanical shaker for 4hrs and filtered. The filtered marc obtained was again added with solvent and the procedure was repeated. The contents obtained after shaking was filter through muslin cloth and the filtrate was concentrated under reduced pressure and control temperature to yield a dark gummy solid. The extract was preserved in a refrigerator at 4^{o}C.[10]

2.4 Phyto Chemical Screening

Chemical tests were carried out using the extract of *Asystasia gangetica, Ficus recemosa* and *Morus indica* for the presence of phyto chemical constituents [11] by standard procedures.

2.5 experimental animals

Healthy adult albino rats of *Wistar* strains (150-200gm) were used in the present study. The animals were caged in clean polypropylene cages under controlled temperature 20-24^{0}c and 12hr light/dark and were fed with standard rat pelleted diet[7] and clean drinking water was made available *ad libitum*. All experiments and protocols of present study were approved by the Institutional Animal Ethical Committee of Sri Padmavathi School of Pharmacy.

2.6 Acute oral toxicity

Animals were starved overnight and divided into 5 groups. The animals were orally fed with extracts separately in increasing dose level of 250, 500, 1000, 1500 and 2000 mg/kg. The animals were observed 24 hours for any mortality as per OECD guidelines.[8]

2.7 experimental induction of diabetes

Animals were allowed to fast for 12hr and after baseline blood glucose estimation they were administered freshly prepared streptozotocin (STZ-60mg/kg b.w.i.p) in 0.1 mol/L cold citrate buffer(p^H 4.5). The STZ treated animals were allowed to drink 5% glucose solution to overcome drug induced hypoglycaemia for 12 hours, after 5 days of development of diabetes, rats with moderate diabetes having persistence glycosuria and hyperglycaemia (blood glucose>250mg/dl) were considered as diabetic and were used for further experiments.[12]

2.8 experimental designs

Animals were divided into five groups, consisting of a minimum of six animals each:

Group 1: Control rats (10ml/kg NS)

Group 2: Diabetic control

Group 3: Diabetic rats administered
AGLE (50 mg/kg) +
FRLE (75 mg/kg) +
MILE (75mg/kg)

Group 4: Non diabetic rats administered
AGLE (50 mg/kg) +
FRLE (75 mg/kg) +
MILE (75mg/kg)

Group 5: Diabetic rats administered
Glibenclamide (5mg/kg).

All the drugs were administered orally and treatment was continued for 28 days. The doses employed for all drugs were within therapeutic range to suit the experimental animal used.

2.9 sample collection for haematological estimations

Blood samples were collected from tip of rat tail and blood glucose levels were estimated using **SD** *CHECK*. The body weight measured during treatment on weekly basis (i.e. 0, 7, 14, 21 and 28 days). Few ml of blood was collected for RBC, WBC, Haemoglobin, $HbA1_{C,}$ Bleeding time(Bt) and Clotting time(Ct) evaluation on before and after the treatment days(28days) from retro-orbital plexus under light ether anaesthesia.

2.10 statistical analyses

Values are expressed as mean ± standard error mean (S.E.M) and analyzed using statistical package for social sciences (SPSS) version 10 using ANOVA followed by Dennett's test. $P<0.05$ were considered as significant.

3. RESULTS AND DISCUSSION

Note: The change% indicates that the change between initial and final of treatments within the same groups. (final day - initial day) / initial day x 100).

Table no 1: ***Phyto chemical screening of plant extracts***

Chemical tests were carried out using the extract of ***Asystasia gangetica*** (Triterpenoids, Flavonoids, Steroids, Alkaloids, Saponins, Carbohydrates, Proteins and Tannins) ***Ficus recemosa*** (Triterpenoids, Glycosides, Steroids, Alkaloids, Carbohydrates, Protein) and ***Morus indica*** (Triterpenoids, Flavonoids, Glycosides, Alkaloids, Carbohydrates, Proteins) for the presence of phyto chemical constituents [(11)] by standard procedures.

Table no 2: Effect of PHP on body weight of experimental rats

Shows the bodyweight of experimental animals on basal and 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days of treatments. The results show no intra group variation in the basal body weight. There were significant reduction of body weight in diabetic control rats (-18.60%) compared to normal control group. The 0^{th} day, the body weight showed no much difference in all groups that is during initial diabetic inductive stage but on 7^{th} day significant reduction of body weight was observed in 2^{nd}, 3^{rd} and 5^{th} groups when compared to control rats, this is due to diabetic induction. However, there was no reduction in 4^{th} group (Non diabetic). The drug treated rats gained significant weight ($p<0.01^{a}$-0.05^{b}) on 14 and 28 days of treatment. However, in this study AGLE+ FRLE + MILE treated group showed a better weight gain (+8.72%) when compared to diabetic control group.

Table no 3: Effect of PHP on fasting blood glucose level of experimental rats

This indicates the level of blood glucose experimental rats on basal and 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days of drug treatment. There was a significant reduction of blood glucose in PHP group (-58.93%) and standard group (-59.73%) compared to diabetic control group(+15.14%). The results show no intra group variation in the basal blood glucose level. The 0^{th} day indicates after 5 days of STZ treatment (i.e., diabetic). The blood glucose levels were found to be decreased upon PHP (164.50±.34) and standard drug gradually during 28 days of treatment

and this result was compared to diabetic control and found to be statistically significant(P<0.01 and 0.05), The non diabetic group showed PHP drug induced hypoglycaemia.

Table no 4: Oral glucose tolerance test (OGTT) on experimental rats

The blood glucose levels of experimental rats after oral administration of glucose(2.5g/Kg). In diabetic control rats the peak increase in blood glucose concentration was observed after 90 min (475.35±3.36) and was found to reduce during 2 h. Rats treated with PHP (164.50±0.34) and glibenclamide (165.98±1.98) showed a significant decrease in blood glucose concentration after 1 h of the treatment when compared with diabetic control rats. The glucose tolerance effect was more pronounced after a 2 h interval.

Table no 5: Effect of PHP on hemoglobin and glycosylated haemoglobin(HbA_1C) of experimental rats

Non diabetic group which was treated with the test dose shown significant change in the HB level (+08.08%) i.e., due to nutrientional support of the PHP, on that of the normal control group (+4.44%) But the diabetic groups which were treated with the PHP +74.86 was gained and showed significant gain in standard (Glibenclamide) drug treated (+47.88%) from its loss. Glycosylated Hemoglobin (%) i.e., HbA_1C indicates that the average blood glucose level of past few months. At the end of the treatment day the diabetic control rat shows the maximum level of HbA_1C (11.62 ± 0.38) compare to normal control (7.21 ± 0.53). The PHP (5.61 ± 0.27) and standard treated (5.48 ± 0.29) showed significant reduction than diabetic control.

Table no 6: Effect of PHP on RBC and WBC counts of experimental rats

RBC and WBC counts were done from initial, 0^{th} day and during final day of treatments also calculated change% that the diabetic control rats showed the declining of RBC (-19.26%) count than the normal control RBC (+2.9%). The PHP treated diabetic group (+14.95%) and standard treated (+20.47%) shows good gaining of RBC count. The only PHP treated non diabetic (+14.95%) indicates the nutritional value of RBC gaining.

This table also reported that the diabetic control rats showed the declining of WBC (-36.85%) count than the normal control WBC (+0.84%). The PHP treated diabetic group (+6.9%) and standard treated (+8.48%) shows good gaining of WBC count. The only PHP treated non diabetic (+1.45%) indicates the nutritional value of WBC gaining.

Table no 7: Effect of PHP on bleeding (BT)time and clotting time(CT) of experimental rats

BT and CT were done from initial, 0^{th} day and during final day of treatments also calculated change% that the diabetic control rats showed the declining of BT (-57.33%) than the normal control (+7.85%). The PHP treated diabetic group (+45.85%) and standard treated (+58.11%) shows good gaining of BT. The only PHP treated non diabetic (+1.95%).

This table also reported that the diabetic control rats showed the decreasing of CT (-13.04%) than the normal control CT (+3.17%). The PHP treated diabetic group (+140.74%) and standard treated (+180.00%) shows good gaining of CT count. The only PHP treated non diabetic (+3.28%) indicates the nutritional value of CT gaining.

Table no 1: Phytochemical Screening of Agle, Frle & Mile.

S.N.	Constituents	Agle	Frle	Mile
1.	Tri terpenoids	+	+	+
2.	Flavonoids	+	-	+
3.	Glycosides	-	+	+
4.	Steroids	+	+	-
5.	Alkaloids	+	+	+
6.	Saponins	+	-	-
7.	Carbohydrates	+	+	+
8.	Proteins	+	+	+
9.	Tannins	+	+	+

(+) Presence of constituents (-) Absence of constituents

Table no 2: Effect of PHP on Body Weight of Experimental Rats.

S.N.	Drug Treatment	Before STZ	After STZ Average body weight (gm)					% Change in wt gain
		Basal B.Wt	0 day	7th day	14th day	21st day	28th day	
1	Control Normal saline (10 ml/kg)	163.40±1.25 [a]	167.50±1.95 [a]	172.50±1.81 [a]	177.50±1.75 [a]	182.50±1.70 [a]	189.50±1.80 [a]	+13.17
2	Diabetic control (STZ-60 mg/kg)	170.50±1.55 [a]	172.33±1.92 [b]	165.33±1.85 [b]	159.50±1.85 [b]	150.50±1.80[b]	140.80±1.77[a]	-18.60
3	Diabetic+ (AGLE+FRLE+MILE)	175.30±1.15 [a]	172.50±1.79 [a]	171.50±1.88 [a]	175.40±1.94 [a]	181.40±1.92 [a]	187.30±1.83 [a]	+ 8.72
4	Non diabetic + (AGLE+FRLE +MILE)	175.20±1.85 [a]	178.20±1.81 [a]	181.50±1.99[b]	184.40±1.87[b]	188.20±1.78[b]	191.31±1.85 [a]	+7.30
5	Diabetic+Glibenclamide (5 mg/kg)	172.70±1.05 [a]	175.50±0.98 [a]	170.50±1.70[b]	175.25±0.92[b]	181.50±1.84[b]	189.50±1.65 [a]	+8.00

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01[a]-0.05[b]) the 3, 4 and 5th groups are compared with control (1) and diabetic control (2).

Table no 3: Effect of PHP on Fasting Blood Glucose Level of Experimental Rats.

S.No	Drug Treatment	Basal FBG	Average blood glucose(mg/dL)					% changes in FBG
			0th day	7th` day	14th day	21st day	28th day	
1	Control (Normal saline 10ml/Kg)	101.05±1.07[a]	103.05±1.57[a]	103.21±1.80[a]	105.21±1.91[a]	104.24±1.88[a]	105.69±1.78[a]	+2.56
2	Diabetic control (STZ-60mg/kg)	107.15±1.67[a]	295.83±1.76[a]	292.25±1.67[a]	322.05±1.88[a]	331.05±2.34[a]	340.61±2.35[a]	+15.14
3	Diabetic + (AGLE+FRLE +MILE)	100.05±1.49[a]	290.50±1.78[a]	212.40±2.23[a]	155.20±1.81[b]	125.30±1.98[a]	119.30±1.8[a]	-58.93
4	Non diabetic + (AGLE+FRLE +MILE)	109.35±1.00[a]	107.40±1.91[a]	108.20±1.72[b]	104.30 ±1.83[a]	100.20 ±1.74[a]	96.50±1.61[a]	-10.15
5	Diabetic + Glibenclamide (5mg/kg)	114.05±1.57[a]	301.31±2.3[a]	289.32 ±2.1[a]	194.61±1.81[a]	146.33±1.85[b]	121.34±1.94[b]	-59.73

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01[a]-0.05[b]) the 3, 4 and 5th groups are compared with control (1) and diabetic control (2).

Table no 4: Oral Glucose Tolerance Test (Ogtt) On Experimental Rats After 28 Days.

S.No	Treatments	0^{th}min (Initial BG)	30^{th} min	60^{th} min	90^{th} min	120^{th} min	150^{th} min	180^{th} min	% Tollarability
1	**Control Normal saline (10 ml/kg)**	105.69±1.78[a]	164.09±1.78[a]	136.10±1.08 [a]	129.00±1.22[a]	120.45±1.68[a]	112.78±1.49 [a]	118.28±1.59 [a]	+ 55.26
2	**Diabetic control (STZ-60 mg/kg)**	340.61±2.35[a]	21.09±1.05[a]	444.86±2.55[a]	475.35±3.36[a]	460.70±1.48[a]	445.23±1.39[a]	432.76±2.33[a]	+ 39.56
3	**Diabetic + (AGLE+FRLE+ MILE**	119.30±1.80[a]	156.00±1.98[a]	164.50±0.34[a]	144.25±1.45[a]	139.88±1.78 [a]	131.98±1.08 [a]	125.54±1.19[a]	+ 37.89
4	**Non diabetic + (AGLE+FRLE +MILE)**	96.50±1.6I [a]	110.22±1.81[a]	140.56±0.08[a]	131.98±1.65[a]	122.96±2.08[a]	114.38±2.81[a]	109.10±2.09[a]	+ 45.67
5	**Diabetic+Glibenclamide (5 mg/kg)**	127.34±1.94[b]	159.00±1.09[b]	165.98±1.98[b]	152.76±1.45[b]	140.86±2.16[b]	131.23±1.67[b]	125.09±2.04[b]	+ 30.34

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01[a]-0.05[b]) the 3, 4 and 5^{th} groups are compared with control (1) and diabetic control(2).

% TOLLARABILITY = ((MAX BG – 0^{th} MINUTES BG) / 0^{th} MINUTES BG) X 100

Table No 5: Effect of PHP on Hemoglobin and Glycosylated Haemoglobin of Experimental Rats.

S.No	Drug Treatment	Hemoglobin g/dL				Glycosylated Hemoglobin(%)HbA_1C			
		Before STZ	Post treatment			Pre treatment Before STZ	Post treatment		
			Initial (0 day)	Final (28^{th} day)	% change		0 Day	28^{th} Day	% change
1	Control (Normal saline 10ml/Kg)	12.59±0.98[a]	12.84±0.65[a]	13.41±0.73[a]	+4.44	6.06 ± 0.18[b]	6.82 ± 0.34[b]	7.21 ± 0.53[b]	+5.72
2	Diabetic control (STZ-60mg/kg)	12.64±0.60[a]	5.47±0.97[a]	4.23±0.93[b]	-22.67	4.96 ± 0.19[a]	11.52 ± 0.98[b]	11.62 ± 0.38[a]	+0.87
3	Diabetic + (AGLE+FRLE+ MILE)	12.04±0.65[a]	7.32±0.88[a]	12.80±0.74[a]	+74.86	5.78 ± 0.38[a]	10.48 ± 0.59[a]	5.61 ± 0.27[a]	-46.47
4	Non diabetic (AGLE+FRLE+ MILE)	11.84±0.65[a]	12.01±0.67[a]	12.98±0.99[b]	+08.08	5.64 ± 0.98[b]	6.00 ± 0.36[b]	6.11 ± 0.23[b]	+1.83
5	Diabetic + (Glibenclamide 5mg/kg)	10.87±0.75[a]	8.71±0.97[a]	12.88±0.67[a]	+47.88	5.26 ± 0.32[a]	10.97 ± 0.39[a]	5.48 ± 0.29[a]	-50.05

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01^a-0.05^b) the 3, 4 and 5^{th} groups are compared with control (1) and diabetic control (2).

Table No 6: Effect of PHP on RBC and WBC Counts of Experimental Rats.

S.No	Drug Treatment	RBC mm^3 blood (Lakhs)				WBC /(mm3 blood)			
		Before STZ	Initial (0 day)	Final (28^{th}day)	%changes	Before STZ	Initial (0 day)	Final (28th day)	%changes
1	Control (Normal saline 10ml/Kg)	81.72±0.32[a]	82.8±0.50[a]	85.20±0.62[a]	+2.90	8002±34[a]	7775±5.51[a]	7840±6.60[b]	+0.84
2	Diabetic control (STZ-60mg/kg)	89.89±0.76[a]	73.2±0.75[a]	59.10±0.82[b]	-19.26	7950±65[a]	6350±3.29[b]	4010±4.52[a]	-36.85
3	Diabetic + (AGLE+FRLE+ MILE)	82.03±0.20[a]	68.9±1.20[a]	79.20±1.75[b]	+14.95	8234±76[a]	6120±2.51[b]	6542±5.42[a]	+6.90
4	Non diabetic + (AGLE+FRLE+ MILE)	90.02±0.54[a]	93.2±1.75[a]	101.1±0.95[a]	+8.48	8054±23[a]	7950±5.41[a]	8065±5.20[a]	+1.45
5	Diabetic + (Glibenclamide 5mg/kg)	92.10±0.21[a]	59.1±1.20[a]	71.20±1.50[b]	+20.47	8101±47[a]	6250±4.21[b]	6780±4.48[b]	+8.48

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01^{a}-0.05^{b}) the 3, 4 and 5^{th} groups are compared control (1) and diabetic control (2).

Table no 7: Effect of PHP on Bleeding Time and Clotting Time of Experimental Rats.

S.No	Drug Treatment	Bleeding time(seconds)				Clotting time(minutes)			
		Before STZ	Initial (0^{th}day)	Final (28^{th} day)	%changes	Before STZ	Initial (0 day)	Final (28th day)	%changes
1	Control (Normal saline 10ml/Kg)	36.42 ± 03[a]	35.65 ± 1.25 [a]	38.45 ± 2.52 [a]	+7.85	3.98 ± 1.09[a]	3.15 ± 0.05 [a]	3.25 ± 0.09 [a]	+3.17
2	Diabetic control (STZ-60mg/kg)	37.89 ± 34 [a]	25.05 ± 2.25 [a]	10.69 ± 1.56 [a]	-57.33	3.76 ± 0.06 [a]	1.15 ± 0.07 [a]	1.00 ± 0.08 [a]	-13.04
3	Diabetic +(AGLE+FRLE+MILE)	39.12 ± 46 [a]	24.67 ± 0.56 [a]	35.98 ± 2.12 [a]	+45.85	3.09 ± 2.00 [a]	1.35 ± 0.05 [a]	3.25 ± 0.07 [a]	+140.74
4	Nondiabetic +(AGLE+FRLE +MILE)	33.53 ± 97[b]	36.84 ± 1.11 [b]	37.56 ± 1.25 [b]	+1.95	4.09 ±1.69[b]	3.05 ± 0.06 [b]	3.15 ± 0.05 [b]	+3.28
5	Diabetic + Glibenclamide 5mg/kg)	37.58 ± 46 [a]	24.09 ± 2.14 [a]	38.09 ± 1.17 [a]	+58.11	4.01 ± 1.14[a]	1.25 ± 0.0 [a] 3	3.50 ± 0.03 [a]	+180.00

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01^{a}-0.05^{b}) the 3, 4 and 5^{th} groups are compared with control (1) and diabetic control (2).

8. CONCLUSION

I may conclude from the above experimental report stated, their occurs selective decrease in the hyperglycaemic state after the administration of AGLE +FRLE +MILE(PHP)reduces the severity of oxidative stress and acuity of hyperglycaemia (induced by STZ), a process that it closely linked to glucose oxidation and formation of free radicals. The results suggested that PHP has more favourable effect on oxidants in STZ – induced diabetic rats compared with Glibenclamide as well as regeneration of β-cells of pancreas. The present study suggests that PHP can be successfully utilised for the management of diabetes due to their hypoglycaemic action. The natural herbal extracts are with Nutraceuticals and Herbominerals which help in the growth and development of the tissues and organs followed by weight gain. In this study PHP treated group showed a better weight gain. Further studies on the nature of functional group involved and isolation of active constituents would enlighten the exact mechanism and thus help to rationalize their use in the treatment diabetes more effectively.

9. REFERENCES

1. American Diabetes Association, Diagnosis and classification of Diabetes mellitus. (2005); 537-39.
2. Omonkhelin J Owolabi, Fabian C Amaechina, Mercy Okoro, Effect of Ethanol Leaf Extract of Newboulda Laevis on Blood Glucose Levels of Diabetic Rats, Tropical Journal of Pharmaceutical Research, 2011; 10(3): 249-54.
3. E. E. Jerald, S. B. Joshi, D. C. Jain, S. Edwin Biochemical Evaluation of the Hypoglycemic Effects of Extract and Fraction of *Cassia fistula* Linn. in Alloxan-induced Diabetic Rats. Indian J Pharm Sci., 2013; 75(4): 427–34.
4. Rahman M Hafizur, Randa Babiker, Sakina Yagi , Sidra Chishti, Nurul Kabir, M Iqbal Choudhary The antidiabetic effect of Geigeria alata is mediated by enhanced insulin secretion, modulation of b-cell function, and improvement of antioxidant activity in streptozotocin-induced diabetic rats. Journal of endocrinology, 2012; 214: 329-35.
5. Wild S, Roglic G, Green A, Sicree R, King H. "Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030". Diabetes Care, 2004; 27(5): 047–53.
6. A Doss* and SP Anand, Free Radical Scavenging Activity of Solanum trilobatum Linn. on Alloxan - Induced Diabetic Rats Biochemistry & Analytical Biochemistry, 2012; 1(6): 1-6.
7. Carole Nelson. A healthy rat diet, www.lilratscal.com, 2007.

8. OECD Guideline for testing of chemicals, Acute Oral Toxicity- Up and Down Procedure, 2001.
9. K.D. Tripathi Essentials of medical pharmacology third ed., Jaypee brothers, New Dellhi,1995; 321-22.