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EFFECT OF INGREDIENTS OF A LOCAL ANTIDIABETIC FORMULATION IN ENHANCING THE ANTIDIABETIC ACTIVITY OF *SALACIA RETICULATA* IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT: *Salacia reticulata*, used in traditional and local medicines in Western Ghats of Southern India for the treatment of hyperglycaemia, is widely used for its sugar lowering activity. Accordingly, the present study was designed to investigate the possible actions of ethanolic extract of roots of Salacia reticulate (EESR) and an ethanolic extract of local formulation (EELF) made by mixing roots of *Salacia reticulata* along with five other drugs, each one in equal ratios, namely heartwood of *Pterocarpus marsupium*, stem of *Tinospora cordifolia*, fruits of *Phyllanthus emblica*, roots of *Vetiveria zizanioides*, and heartwood of Acacia catechu, on sugar lowering effect of diabetic rats. In the first set of experiments, hypoglycaemic effects of oral administration at various doses (50 and 100mg/kg) of the both extracts (EESR & EELF) were examined in normoglycaemics, glucose-induced hyperglycaemic rats. Optimum effect was observed in all groups of animals with a dose of 100mg/kg of the extracts (EESR & EELF). In other part of the study, both extracts (EESR & EELF) of 100mg/kg was given to alloxan induced hyperglycaemic rats of different groups and blood sugar was recorded at an interval of 30 mins each for upto 360 mins to evaluate the intraday effect of EESR & EELF on blood glucose level. The last set of experiments was done by giving twice in the morning and evening the EESR & EELF orally at a dose of 100mg/kg for 9 days to check the inter day effect of extracts (EESR & EELF) on blood glucose of diabetic rats. Both the intraday study and the interday study have shown a significant decrease in blood glucose level of the diabetic and normal rats with the help of extracts. Both, intraday and interday study, have shown a rather more decrease in blood glucose level of both diabetic and normal rats, when treated with EELF, signifying the synergistic study of the other ingredients of the formulation on the blood lowering effect of *Salacia reticulate.*

INTRODUCTION: Diabetes mellitus represents a group of diseases of heterogeneous etiology, characterized by chronic hyperglycaemia and other metabolic abnormalities, which are due to deficiency of insulin effect.

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After a long duration of metabolic derangement, specific complications of diabetes like retinopathy, nephropathy and neuropathy may occur. Depending on the severity of the metabolic abnormality, diabetes may be asymptomatic, or may be associated with symptoms (thirst, polyuria, and weight loss), or may progress to ketoacidosis and coma [1].

In many traditional schools of medicine it is claimed that a balanced modulation of several targets can provide a superior therapeutic effect and decrease in side effect profile compared to a single

action from a single selective ligand, especially in the treatment of certain chronic and complex diseases such as diabetes and obesity. Diabetes and obesity have a multi-factorial basis involving both genetic and environmental risk factors. A wide array of medicinal plants and their active constituents play a role in the prevention and treatment of diabetes [2]. 152 million people are suffering from diabetes in India and the number is increasing every year. About 148 plants of 50 families reported to have hypoglycemic activity [3].

In Kerala, the plants like *Salacia reticulata, Pterocarpus marsupium, Tinospora cordifolia, Phyllanthus emblica, Vetiveria zizanioides* and *Acacia catechu* are used extensively in one form or other by the local people for treating the diabetic disorders. Very little scientific work has been done so far on these plants especially on *Salacia reticulata.*

Salacia Reticulata, also known as Akanayakam, is a climbing shrub with blackish branches. *Salacia reticulata's* root bark extract when administered orally to streptozotocin induced diabetic rats, showed hypoglycemic activity at all doses tested. The maximum percentage decrease in plasma glucose was observed between 1 and 5 hours following administration [4]. Salacia roots have been used in Ayurvedic medicine for diabetes and obesity since antiquity, and have been extensively consumed in Japan, the United States and other countries as a food supplement for the prevention of obesity and diabetes. Recent pharmacological studies have demonstrated that Salacia roots modulate multiple targets: peroxisome proliferator-activated receptor-alpha-mediated lipogenic gene transcription, angiotensin II/angiotensin II type 1 receptor, alpha-glucosidase, aldose reductase and pancreatic lipase. These multi-target actions may mainly contribute to Salacia root-induced improvement of type 2 diabetes and obesity-associated hyperglycemia, dyslipidemia and related cardiovascular complications seen in humans and rodents.

The results of bioassay-guided identification indicate that mangiferin, salacinol, kotalanol and kotalagenin 16-acetate are at least in part responsible for these multi-target regulatory activities of Salacia roots. The evidence suggests that this unique traditional medicine fulfills a multiple-target strategy in the prevention and treatment of diabetes and obesity. Although toxicological studies have suggested minimal adverse effects of the herbal medicine in rodents, a clinical trial is crucial to further confirm the safety of Salacia roots. In addition, further mechanistic studies are necessary in order to allow a better understanding of how use of Salacia root may interact with other therapeutic interventions [5].

Pterocarpus marsupium, also known as Vijayasar, is a moderate sized to large deciduous tree, upto 30 meters high. Extract of heartwood showed statistically hypoglycaemic action in fasting rabbits 3 and 5 hours after oral administration. No harmful effect was noticed in doses which showed hypoglycaemic action. Alcoholic extract of stem significantly lowered blood sugar and improved glucose tolerance of rabbits. Clinical trials on heartwood (extract, decoction, powder and infusion) showed encouraging hypoglycemic effects in a number of diabetic patients. Considerable reduction in sugar levels in blood and urine were observed [6].

Tinospora cordifolia is a large, glabrous, deciduous, climbing shrub. A compound preparation in which *Tinospora cordifolia* was one of the constituents was tried in 20 patients of diabetes. It was concluded that the drug provides a total beneficial therapy in all types of diabetes and tends to increase insulin secretion from islets of pancreas. Efficacy of a proprietary herbal preparation in which *Tinospora cordifolia* was one of the constituents was evaluated on 28 cases of persistant post prandial hyperglycaemia. After 12 weeks of treatment a persistent fall in fasting and post prandial blood glucose levels was recorded.

A clinical trial was taken on 25 patients of type II diabetes to study the adjuvant effect of a herbo-mineral proprietary preparation in which *Tinospra cordifolia* was one of the constituents. The drug contained 19 ingredients in which 13 were minerals and 6 were herbals. The herbomineral preparation showed improvement in glycaemic parameters, viz. fasting blood sugar levels, post lunch blood sugar levels and Fructosamine levels. It also improved

fasting and post prandial hyperglycaemic control. This indicates that the proprietary medicine is useful adjuvant in poorly controlled type-II diabetes [6].

Phyllanthus emblica, also known as Amla, is a small or medium sized, deciduous tree. A clinical study was conducted on 25 patients of type II diabetes with a herbomineral proprietry preparation, of which fruit of *Phyllanthus emblica* was one of the constituents. The patients were administered 2 tabs 3 times a day in addition to regular sulphonylureas over a period of 6 weeks. It showed improvement in glycaemic parameters viz. fasting blood glucose, post lunch blood glucose and fructosamine levels. This indicates that the compound preparation can be a useful adjuvant in poorly controlled type II diabetes [6].

Vetiveria zizanioides, also known as Khass, is a perennial herb upto 2 meters height. Vetiveria zizanioides, an aromatic plant commonly known as vetiver has been used for various ailments. The essential oil of Vetiver root has been shown to possess antioxidant activity [7].

Acacia catechu is a moderate sized deciduous tree upto 3m in height. Acacia extract when fed to normal young albino rats, produced marked hypoglycemic activity [8]. Present study is meant to identify the antidiabetic activity of the plant *Salacia reticulate* and to find out the synergistic activity of other ingredients in glucose reducing activity of Salacia reticulate.

MATERIAL AND METHODS:

Plant material:

Salacia reticulate has been procured from Payneer Ayurvedical, Irinjalakuda, Thrissur, Kerala, India. Pterocarpus marsupium, Phyllanthus embellica, Vetiver zizanioides, Acacia catuchu were collected from Kodivalapalapi Gardens, Vellenjor, Thrissur, Kerala, India and Thumboormuzhy Garden, District Tourism Promotion Council, Thumboor, Thrissur, Kerala, India. Tinospora cordifolia was collected from Pullukara House, Kottenloor, Irinjalakuda, Thrissur, Kerala, India. All the drugs were collected at daytime in the month of May and June 2009. The plant has been identified by Dr. H.B. Singh, Scientist and Head of Raw Material Herbarium and Museum, National Institute of Scientific Communication and Information Resources (NISCAIR), New Delhi, India. Dried specimens were deposited in the Herbarium of NISCAIR with reference number NISCAIR/RHMD/Consult/-2009-10/1393/195. The plant materials (Acacia catechu heartwood, Phyllanthus embellica fruit, Pterocarpus marsupium heartwood, Salacia reticulate root, *Tinospora cordifolia* stem, Vetiveria zizanioides root) were dried under shade at 25°C and grinded with a cutter and then blender. Sieving was done with sieves no. 40 & 60. The powdered drugs were kept in air proof plastic boxes according to their particle size till the time of use.

Preparation of extracts:

The powdered *Salacia reticulate* root was extracted in Soxhlet apparatus with 95% ethanol at a temperature of 60°C for 48 hours. The residue (impurities) was removed by filtration and the alcoholic extract was concentrated in a rotary evaporator under reduced pressure at a temperature of 50°C. Local formulation (EELF) was made by mixing roots of *Salacia reticulata* along with five other drugs, each one in equal ratios, namely heartwood of *Pterocarpus marsupium*, stem of *Tinospora cordifolia*, fruits of *Phyllanthus emblica*, roots of *Vetiveria zizanioides*, and heartwood of Acacia catechu. This mixture is used for the preparation of extract in the same as of Salacia reticulate. The dried materials (EELF & EESR) were kept in air-tight container in refrigerator for further use.

Animals:

Adult Wister rats of either sex weighing 150-250 gm were used for experiments. All rats were kept in individual cages located in the animal house of Department of Pharmacology, Ram-Eesh Institute of Vocational and Technical Education, Greater Noida, Uttar Pradesh, India, at room temperature of 20-22°C at 45-55% relative humidity for 12 hour, each of dark and light cycle. They were fed with a standard diet and water ad libitum. The rats were kept in these conditions for 7 day period of adaptation prior to start of the experiment. They were fasted overnight, 6-10 hrs, before the experiments but allowed free access to water. The experiment protocol has been approved by

IAEC/CPCSEA with registration no: 385/CPCSEA with project no. 2012/PhD/06.

Toxicity study (LD_{50}):
Acute toxicity studies were conducted as per internationally accepted protocol drawn under OECD guidelines [9, 10]. Healthy adult wistar albino rats (150-250 gm) of either sex, starved overnight and were divided into four groups (n=6) and were orally fed with the extracts in increasing dose levels of 50, 100, 200, 500mg/kg body weight. The rats were observed continuously for 2 hrs for behavioural, neurological and autonomic profiles and continuously for 3 weeks for any lethality after administration[11]. No untoward effect has been found to present after the dosage in any of the groups of rats for the first two hours and also for 3 weeks continuous supervision

Dose selection:
EESR and EELF extracts were prepared according to their effective doses (ED_{100}). They were well mixed with the addition of 1% of carboxymethycellulose (Suspending agent) till the stable and homogeneous suspension is formed.

Oral glucose tolerance test:
Fasted normoglyceamic rats were divided into six groups of six animals each.

Group A, serving as control, received only vehicle (Carboxymethylcellulose in distilled water, 1% w/v) orally in volume of 5ml/kg.

Group B received glibenclamide as reference drug (300µg/kg) suspended in vehicle.

Group C & D received a EESR at a dose of 50mg/kg and 100mg/kg respectively.

Group E & F received EELF at a dose of 50mg/kg and 100mg/kg. The rats of all the groups were given glucose (2gm/kg), 30 min after glibenclamide and test extract administration. Measurement was done 1 hr, 2 hr and 6 hr after dosing.

Induction of non-insulin dependent diabetes mellitus (NIDDM): Rats were made diabetic by a single intraperitonial injection of alloxan monohydrate in normal saline at a dose of 75mg/kg body weight. Then three days later blood samples were collected and glucose levels were determined to confirm the development of diabetes. The rats with blood glucose level above 250mg/dl were considered to be diabetic and were used in the experiment.

Antidiabetic Study:
Intraday Antidiabetic study:
Rats were made diabetic by a single intraperitonial injection of alloxan monohydrate (R K Enterprises, Meerut) in 5% (w/v) water for injection at a dose of 75mg/kg. Then, 3 days later blood samples were collected and glucose levels were determined to confirm the development of diabetes. The rats with blood glucose level more than 250mg/dl were considered to be diabetic and were used (Reference) in the experiment. Four groups were made and each group contains six animals each. Group A, serving as diabetic control, received only vehicle (Carboxymethylcellulose in distilled water, 1% w/v) orally in volume of 5ml/kg. Group B received glibenclamide as reference drug (300µg/kg) suspended in vehicle. Group C received EESR at a dose 100mg/kg. Group D received EELF at a dose of 100mg/kg. The effects of administration of extracts on diabetic rats were observed by measuring blood glucose level at every half an hour starting from 0 min. to 360 min.

Interday Anti-diabetic study:
Fasted rats were divided into five groups of six animals each. First group of animals received only vehicle (Carboxy methylcellulose in distilled water, 1% w/v) orally in a volume of 5ml/kg and served as control. Group B was made diabetic by a single intraperitonial injection of alloxan monohydrate (R K Enterprises, Meerut) in 5% (w/v) water for injection at a dose of 75mg/kg. Then, 3 days later blood samples were collected and glucose levels were determined to confirm the development of diabetes.

The rats with blood glucose level more than 250mg/dl were considered to be diabetic and were used (Reference) in the experiment. Group C were made diabetic by the same procedure as of group B and were given Glibenclamide suspended in the vehicle as reference drug (300µg/kg) orally. Group

D were also made diabetic in the same way as of group B and were given *Salacia reticulata* extract (EESR) (100mg/kg) suspended in the vehicle. Group E was given extract of local formulation (EELF) (100mg/kg) orally twice for 9 days. The effects of administration of extracts in normal and diabetic rats were observed by measuring fasting blood glucose on 0, 3, 6 and 9 days of extract administration.

Statistical analysis:

Values are presented as means ± S.E.M. Statistical differences between the treatments and the controls were tested by one-way analysis of variance (ANOVA) followed by the student-Newman-Keuls test using the "Origin" computer software. A difference in the mean values of $p < 0.05$ or less was considered to be statistically significant.

Glucose Estimation:

Blood samples were collected for glucose level estimation by glucose oxidase-peroxidase reactive strips and a glucometer (Accu check, Roche Diagnostics, USA). Blood glucose level was estimated in mg/dl.

RESULTS:

Effect of Glibenclamide, Ethanolic extract of Salacia reticulate and Ethanolic extract of Local Formulation on blood glucose level in normoglyceamic rats (Oral glucose tolerance test):

Vehicle, glibenclamide and two different doses (50mg/kg and 100mg/kg) of Salacia reticulata and Formulation were given half an hour before oral administration of glucose (2gm/kg) on six groups containing 6 normoglyceamic rats each. Blood glucose level for measured is summarized in **Table 1**. The blood glucose levels of the normoglycaemic rats reached a peak at 1h after the real administration of glucose and gradually decreased to the preglucose load level.

In glucose induced hyperglycaemic rats the extract at dose of 50mg/kg and 100mg/kg showed significant effect, with blood glucose level dropping to 20% from that of the control. At 100mg/kg dose of the extract most potent reduction was observed with the blood glucose level reducing considerably. It therefore appears that 100mg/kg of the extract of *Salacia reticulata* and local formulation is the most effective dose of oral glucose tolerance test of the normoglyceamic rats. Glibenclamide prevented the drastic increase of blood glucose 1hr after the glucose loading and reduced the level even below the normal values at 2 hr and 6hr after the glucose loading.

The extracts of *Salacia reticulata* and local formulation could increase insulin concentration in a time and dose dependent manner in glucose loaded rats when compared to control. On the other hand, glibenclamide showed more pronounced insulinotropic activity in glucose loaded rats.

TABLE 1: EFFECT OF GLIBENCLAMIDE, ETHANOLIC EXTRACT OF SALACIA RETICULATE AND ETHANOLIC EXTRACT OF LOCAL FORMULATION ON BLOOD GLUCOSE LEVEL IN NORMOGLYCEAMIC RATS (ORAL GLUCOSE TOLERANCE TEST)

S. No.	Group	Dose (mg/kg)	Mean blood glucose concentration ± S.E.M (mg/dl)			
			0 hr	1 hr	2 hr	6 hr
A	Control		119.14 ± 3.88	138.24 ± 4.25	140.82 ± 4.28	125.21 ± 6.23
B	Glibenclamide	300µg/kg	122.34 ± 3.44	107.23 ± 4.85*	94.45 ± 2.58***	80.35 ± 4.35***
C	Sal. Ret.(EESR)	50mg/kg	121.22 ± 2.22	131.34 ± 3.54	118.24 ± 2.43	125.32 ± 4.32
D	Sal. Ret.(EESR)	100mg/kg	119.25 ± 1.54	125.43 ± 2.38	110.25 ± 1.33*	122.34 ± 3.26
E	Form. (EELF)	50mg/kg	117.29 ± 3.24	129.32 ± 3.22	120.43 ± 3.42	123.32 ± 2.34
F	Form. (EELF)	100mg/kg	118.43 ± 2.12	128.34 ± 2.23	103.32 ± 4.32**	128.34 ± 3.42

S.E.M: mean standard error, *$p < 0.05$ significant from the control animals, **$p < 0.01$ significant from the control animals, ***$p < 0.001$ significant from the control animals

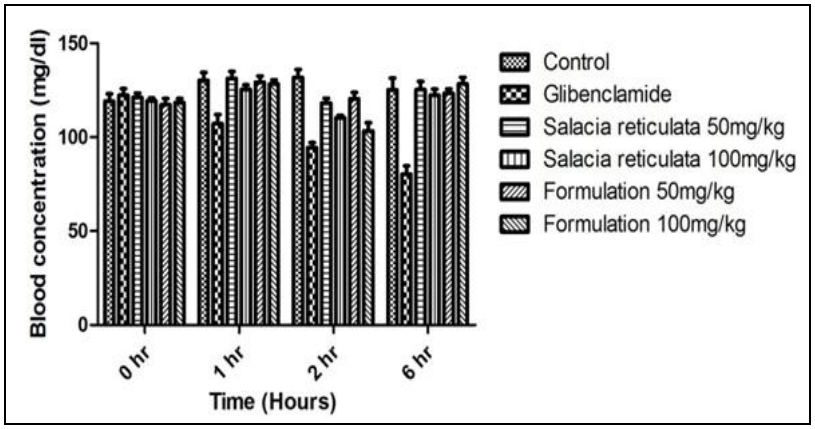

FIG.1: EFFECT OF GLIBENCLAMIDE, ETHANOLIC EXTRACT OF SALACIA RETICULATE AND ETHANOLIC EXTRACT OF LOCAL FORMULATION ON BLOOD GLUCOSE LEVEL IN NORMOGLYCEAMIC RATS (ORAL GLUCOSE TOLERANCE TEST)

Intraday effect of Glibenclamide, Ethanolic extract of Salacia reticulate and Ethanolic extract of Local Formulation on blood glucose level in alloxan induced hypoglycaemic rats:

Intraday effect of extracts of the drug and the formulation in diabetic animals was studied using alloxan induced diabetic rats. As shown in T**able 2** both the extracts (EESR & EELF)shown maximum antidiabetic activity between 90 min. to 120 min. However more pronounced antidiabetic effect was seen by formulation, in comparison to single drug extract, at 90min. where the blood glucose was significantly reduced upto 57.4% of the control. Glibenclamide showed more pronounced antidiabetic activity in alloxan induced diabetic rats than any other extract, inhibiting the blood glucose level dropping to 64.3% from that of control at 120 min.

TABLE 2: INTRADAY EFFECT OF GLIBENCLAMIDE, ETHANOLIC EXTRACT OF SALACIA RETICULATE AND ETHANOLIC EXTRACT OF LOCAL FORMULATION ON BLOOD GLUCOSE LEVEL IN ALLOXAN INDUCED HYPOGLYCAEMIC RATS

Sr. No.	Groups	Mean blood glucose concentration ± S.E.M. (mg/dl)					
		0 min	30 min	60 min	90 min	120 min	360 min
A	Diabetic Control	297.52±2.54	280.54±6.51	318.58±1.53	319.63±10.28	284.53±15.89	311.28±3.24
B	Gliben clamide	304.45±2.57	302.53±22.55	299.82±21.32	195.54±9.50***	102.74±10.46***	120.52±7.43***
C	Salacia Extract	287.25±2.56	286.25±13.24	187.52±12.52***	150.45±25.26***	185.18±15.62***	282.54±1.12
D	Form. Extract	307.65±3.34	298.22±4.45	218.75±1.50***	121.56±6.43***	168.52±17.55***	298.46±2.34

S.E.M: mean standard error, *$p < 0.05$ significant from the control animals, **$p < 0.01$ significant from the control animals, ***$p < 0.001$ significant from the control animals

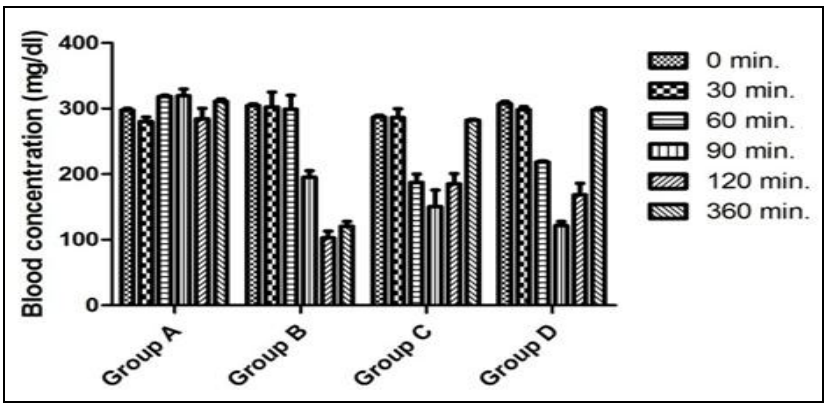

FIG. 2: INTRADAY EFFECT OF GLIBENCLAMIDE, ETHANOLIC EXTRACT OF SALACIA RETICULATE AND ETHANOLIC EXTRACT OF LOCAL FORMULATION ON BLOOD GLUCOSE LEVEL IN ALLOXAN INDUCED HYPOGLYCAEMIC RATS

Inter day effect of Glibenclamide, Ethanolic extract of *Salacia reticulate* and Ethanolic extract of Local Formulation on blood glucose level in alloxan induced hypoglycaemic rats

As shown in **Table 3**, the blood glucose levels of diabetic control rats were significantly higher than those of the normal control rats during the course of experiment. Chronic administration of extracts and glibenclamide during 9 days induced an antihyperglyceamic effect in all rats and reversed the permanent hyperglycaemia caused by alloxan. The antidiabetic effect of EELF at dose of 100mg/kg was more than the EESR at the same dose. The highest reduction in the blood glucose was observed on the 9th day with Glibenclamide, Plant Extract (EESR) and Formulation Extract (EELF) showing 67.9%, 55.2% and 60.3% respectively.

TABLE 3: INTER DAY EFFECT OF GLIBENCLAMIDE, ETHANOLIC EXTRACT OF *SALACIA RETICULATE* AND ETHANOLIC EXTRACT OF LOCAL FORMULATION ON BLOOD GLUCOSE LEVEL IN ALLOXAN INDUCED HYPOGLYCAEMIC RATS

Sr. No.	Group	Dose	Mean blood glucose concentration ± S.E.M. (mg/dl)			
			1st day	3rd day	6th da	9th day
A	Normal Control		85.53±5.62	86.83±5.78	94.34±3.56	86.67±2.53
B	Diabetic Control	75mg/kg	86.83±4.33	296.67±12.24	311.84±9.10	298.56±14.95
C	Standard	300µg/kg	89.56±1.96	276.86±8.17	115.46±4.29***	95.83±3.75***
D	Extract	100mg/kg	95.17±4.16	289.17±16.09	166.33±3.13***	133.67±7.35***
E	Formulation	100mg/kg	101.54±3.22	310.28±10.13	142.15±7.97***	118.67±7.60***

S.E.M: mean standard error, *$p < 0.05$ significant from the control animals, **$p < 0.01$ significant from the control animals, ***$p < 0.001$ significant from the control animals

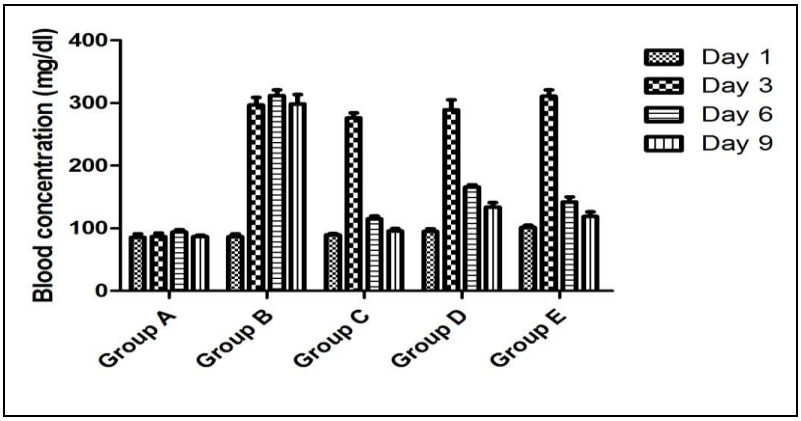

FIG. 3: INTER DAY EFFECT OF GLIBENCLAMIDE, ETHANOLIC EXTRACT OF *SALACIA RETICULATE* AND ETHANOLIC EXTRACT OF LOCAL FORMULATION ON BLOOD GLUCOSE LEVEL IN ALLOXAN INDUCED HYPOGLYCAEMIC RATS

DISCUSSION: The problems of currently available pharmacological agents for control of blood glucose have stimulated research on novel antidiabetic agents with different mechanism of action. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future. Earlier studies have shown the antidiabetic activity of Salacia reticulate when it was administrated to normoglyceamic and hypoglycaemic rats [12]. The present study aimed at further investigating the hypoglycaemic and antidiabetic activity of *Salacia reticulate* and effect of other ingredients on antidiabetic activity of *Salacia reticulata* by forming a formulation containing six ingredients including *Salacia reticulata*. The study signified the synergistic activity of the other ingredients of the formulation on the blood lowering effect of *Salacia reticulate*.

Alloxan a beta-cytotoxin induces chemical diabetes (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting cells of the pancreas. This damages a large number of beta-cells, which paves the ways for the decreased utilization of glucose by the tissue [13]. It is well established that sulphonylureas produces hypoglycaemia by increasing the secretion of

insulin from pancreas and these compounds are active in mild alloxan induced diabetes. But they are inactive in intense alloxan diabetes [14]. EESR and EELF have shown a good antidiabetic activity in alloxan induced diabetic rats.

It appears that 100mg/kg of the extract of salacia reticulata and local formulation is the most effective dose of oral glucose tolerance test of the normoglyceamic rats. Glibenclamide prevented the drastic increase of blood glucose 1hr after the glucose loading and reduced the level even below the normal values at 2 hr and 6hr after the glucose loading. The extracts of *Salacia reticulata* and local formulation could increase insulin concentration in a time and dose dependent manner in glucose loaded rats when compared to control.

Many different mechanisms of action to reduce blood glucose levels with the help of plant extracts were known to us. Some plants exhibit properties similar to the well-known sulfonylurea drugs like glibenclamide; they reduce blood glucose in normoglycaemic animals [15, 16]. Some other plants act like biguanides such as metformin which is an antihyperglycaemic compound; they do not affect blood glucose in normal state [17, 18, 19, 20].

We hypothesized that EESR and EELF could have a sulfonylurea-like mechanism since it decreased blood glucose in normoglycaemic rats such as glibenclamide. Sulfonylurea compounds lower blood glucose in normal and type 2 diabetic animals by stimulating insulin release from pancreatic cells. It is also known that alloxan selectively destroys insulin-secreting cells in the islets of Langerhans and their effects are irreversible [21].

In the present study, the dose of alloxan (75 mg/kg, ip) was selected in order to partially destroy the pancreatic cells. In these conditions, insulin was secreted but not sufficiently to regulate the blood glucose. In alloxan diabetic rats, the hyperglycaemia was permanent and it varied from 276.86 ± 8.17 to 311.84 ± 9.10 mg/dl (ns, n = 6). Glibenclamide is able to decrease the glycaemia in alloxan permanent hyperglycaemic rats. This suggests that the increase of blood glucose obtained with alloxan results from a diminution but not the total abolition of insulin secretion. As well as glibenclamide, EESR and EELF has an antihyperglycaemic effect in alloxan-induced diabetic rats. This shows the activity EESR and EELF similar to Glibenclamide in reducing the hyperglycaemia in rats. This provides the pharmacological basis for the use of *Salacia reticulate* roots and its formulation in traditional medicine to treat diabetes.

The antidiabetic plant extracts in the formulation may involve one or more compounds to decrease blood glucose suggesting that the natural constituents could act separately or synergistically to induce hypoglycaemic effect [22]. We also in our study have found more pronounced antidiabetic effect for the formulation, in comparison to single drug extract at 90min. Intraday study have shown a rather more decrease in blood glucose level of both diabetic and normal rats, when treated with EELF, signifying the synergistic effect of the other ingredients of the formulation on the blood lowering effect of Salacia reticulate. Also, after chronic administration of the extracts at dose of 100mg/kg twice a day for two weeks on diabetic rats, we found that on the 9th day the antidiabetic effect of EELF was more than the EESR at the same dose showing the synergistic activity of ingredients on the antidiabetic activity of Salacia reticulate.

The roots of Salacia contain triterpenoid and flavonoids [23]. Many studies reported the hypoglycaemic activity of terpenoid and flavonoids in diabetic animal models [24]. The EESR could contain flavonoid compounds that may have a good blood glucose reducing activity explaining the antihyperglyceamic activity. It would be interesting to lead further studies to isolate and test the antidiabetic activity of flavonoids from the EESR.

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