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Synergistic Anti-Diabetic Activity of A Polyherbal Extract in Streptozotocin Induced Diabetic Rats

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ABSTRACT

The aim of the present study was to compare the anti-diabetic activity of a polyherbal extract in streptozotocin induced diabetic rats. The ethanolic extracts of leaves of *Alstonia scholaris*, *Centella asiatica*, *Corchorus trilocularis and Morinda pubescens* were prepared. The wistar albino rats were injected with 60 mg/kg of streptozotocin intraperitoneally to induce diabetes. After six weeks of streptozotocin injection, individual extract and polyherbal extract were daily administered at a dose of 200 mg/kg body weight to diabetic rats for a period of 21 days. Blood samples were collected from the retro orbital plexus of the eye and blood glucose level was estimated by glucose oxidase-peroxidase method. The study indicated that polyherbal extract at a dose of 200 mg/kg body weight showed significant decline in blood glucose level as compared to individual extract.The elevated level of SGPT, SGOT in the diabetic controlled group reflected the significant alteration of liver function by STZ induction and was found to be equipotent to Glipizide in restoration of the elevated enzyme levels to normal. The elevated lipid levels (triglyceride and total cholesterol) were restored to near normal by extract for all the estimated parameters. The results of the PHE on treated group were found to restore the significant glycemic level to the near normal level thereby indicating antihyperglycemic activity.

KEYWORDS: Anti-Diabetic, *Alstonia scholaris*, *Centella asiatica*, *Corchorus trilocularis* ,*Morinda pubescens*,Polyherbal extract.

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INTRODUCTION

Diabetes mellitus is a severe metabolic disorder which is indicated by hyperglycaemia due to lack of insulin or the action of insulin on its target tissues or both. It is one of the major public health problem which is now becoming a global epidemic [1] The rising glucose level in blood, in diabetes, results due to combination of unhealthy diet, physical inactivity, defect in insulin secretion in response to food and reduced sensitivity of the target tissues to insulin action [2]. The chronic metabolic disorder which affects about 150 million people of the world is going to increase to 300 million by the year 2025[3].The synthetic oral anti-diabetic drugs and insulin which are being currently used for the control of diabetic complications are effective in controlling the elevated blood glucose levels but they have various side effects and do not control the complications related to diabetes [4].Traditional medicinal plants are being used worldwide for many diabetic complications. Various herbal drugs and minerals have been described in olden traditional literature for the treatment of diabetes mellitus. Herbal drugs are considered to be safe and do not have much side effects compared to synthetic drugs[5]. Therefore, exploring the hypoglycemic potential of medicinal plants has become very important to provide mankind with safer alternative of herbal drugs.

Alstonia scholaris is the tree of family Apocyanaceae, has a promising place in the Ayurvedic system of medicine due to its various medicinal values like antidiabetic, antibacterial, antianxiety, anticancer, hepatoprotective, anti-inflammatory, analgesic effects. The leaves have been used traditionally as folk remedies for the treatment of many diseases including diarrhea, dysentery, and malaria and snake bites. The plant is rich in alkaloids, flavonoids, saponins, steroids, reducing sugars and phenolic compounds which witness the ample amount of medicinal potential of the herb[6].*Centella asiatica*, a perennial herbaceous creeper belongs to the family Umbellifere (Apiceae). It is widely used as a blood purifier as well as for treating high blood pressure, for memory enhancement and promoting longevity. The other reported activities are Anti-inflammatory, Anticancer , Antiulcer, Anxiolytic, Anticonvulsant ac, Antidepressant , Antioxidant a,Immunomodulating a, Cardioprotective , Hepatoprotective , Radioprotective activity, Wound healing, Memory enhancing Activity, Burns, Anti-psoriatic Activity, Antimicrobial Activity, Anti-hyperglycemic and Neuroprotective activity[7-17].*Corchorus trilocularis* L. (Tiliaceae) is one of the most commonly plants in India. The edible leaves of *Corchorus* species are reported to contain some trace mineralsusefultoalleviatemineraldeficienciesofthehumanbody.Theseedsarehotwith a sharp

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taste, alexipharmic, removes tumors, pain, stomach troubles, skin diseases, and scabies. The leaves are reported to prevent cardiovascular disorders and in treatment of diabetes mellitus [18].*Morinda Pubescens* is a flowering shrub-like species belonging to family Rubiaceae. *M. pubescens* is listed as traditional Indian herbal antidiabetics [19]. Traditionally, all parts of the plant especially the leaves and root, are widely used by indigenous people for medicating a full spectrum of ailments and diseases ranging from the topical application to heal wounds, inflammation, gout and stomach pains to oral administration for diabetes, malaria, fever, rheumatism and infectious diseases[20].

Keeping on view of the above effectiveness of the herbals for diabetics, it is proposed to formulate polyherbal extract that is combination of the above plant extract which were studied and the efficacy of the extract is compared with marketed drug Glipizide.The present study has been carried out to determine the synergistic hypoglycemic potential of polyherbal extract in comparison with individual extract and standard drug Glipizide.

MATERIAL AND METHODS

Plant Material

The dried leaves of *Alstonia scholaris, Centella asiatica, Corchorus trilocularis*and *Morinda Pubescens* were purchased from authorized local herbal supplier at Indore (M.P.). The dried leaves of above mentioned were identified and authenticated by Head, Department of Pharmacy, Oriental University, Indore (M.P.).

Preparation of Extracts

The leaves of *Alstonia scholaris, Centella asiatica, Corchorus trilocularis*and *Morinda Pubescens*(1 kg) were air dried and separately coarsely powdered in a mixer. 500 g of each crude drug powder were extracted with the help of soxhlet apparatus [14,21-23].The extracts were concentrated under vacuum, dried at about 60 ˚C and then stored in a refrigerator.

Development of Polyherbal Extract

The polyherbal extract was developed by combining the dried extracts of the plant extracts 100 mg/kg each. The polyherbal formulation was prepared by mixing *Alstonia scholaris, Centella asiatica, Corchorus trilocularis*and *Morinda Pubescens* extract in the ratio of 1:1:1:1 respectively.

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Procurement and selection of animals:

Wistar albino rats of either sex weighing between 100–150 gm of either sex were obtained from central animal house Department of Pharmacy, Oriental University, Indore (M.P.). The animals were stabilized for 1 week; they were maintained in standard condition at room temp; normal light dark cycle. They had been given standard pellet diet and water ad-libitum throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal output.All the animal testing were done under the approval of Institutional Animal Ethical Committee (IAEC) of Oriental College of Pharmacy and Research, Indore. The CPCSEA registration number is IAEC/2019-20/RP-24.

Induction of Diabetes mellitus:

Rats were made diabetic by a single injection of streptozotocin (60 mg/kg, i.p.) prepared in citrate buffer (0.1 M, pH 4.5) after overnight fasting. Blood was drawn from the tail vein 24 h after the injection and the glucose level was estimated by glucose oxidase method by using Accu-Chek Glucometer before and 72 hrs after STZ injection. Animals showed blood glucose level more than 250 mg/dl were selected for further antidiabetic activity [24-25].

Experimental Design:

Animals were divided into eight groups of six rats in each. The LD_{50} value of mixtures was 200 mg per kg body weight. The Plant extracts, Polyherbal extract and standard drug were administered orally once a day for 21 days (Table 1). The blood samples were collected on 1^{st}, 7^{th}, 14^{th}, and 21^{st} days of the treatment, through the tail vein of rats by pricking and were immediately used for the estimation of blood glucose with a glucometer. Results are represented in table 2 and figure 1

Table 1. Treatment of Groups

S. No	Groups	Treatment
1	I : Normal Control	0.5 % w/v sodium CMC in distilled water)
2	II: Diabetic Control	STZ +0.5 % w/v sodium CMC in distilled water
3	III: AS-EE	STZ + Ethanolic extract *Alstonia scholaris* (200 mg/kg body weight)suspended in 0.5 % w/v sodium CMC
4	IV :CA-EE	STZ + Ethanolic extract of dried leaves of *Centella asiatica* (200 mg/kg body weight)suspended in 0.5 % w/v sodium CMC.
5	V : CT -EE	STZ + Ethanolic extract of dried leaves of *Corchorus*

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		trilocularis(200 mg/kg body weight)suspended in 0.5 % w/v sodium CMC
6	VI : MP-EE	STZ + Ethanolic extract of dried leaves of *Morinda Pubescens* (200 mg/kg body weight)suspended in 0.5 % w/v sodium CMC
7	VII :PHE	STZ + Polyherbal Extract (200 mg/kg body weight)suspended in 0.5 % w/v sodium CMC.
8	VIII – Standard Control	STZ + Glipizide (5mg/kg body weight)

Estimation of Biochemical Parameters

The blood samples were collected on the 22nd day from the retroorbital plexus of the rats, serum was separated, and the biochemical estimations of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) [26,27], total protein [28], total cholesterol, and triglyceride were determined. [29, 30].

Statistical Analysis

The results are expressed as mean SD. Statistical evaluation of the data was done by one-way ANOVA followed by Dunnett's *t*-test. The values were considered to be significant when $p < 0.05$.

RESULTS AND DISCUSSIONS

The blood glucose level observations have been recorded in Table 2.The effect of Polyherbal extracts on some biochemical parameters like SGPT, SGOT, total protein, triglyceride, and total cholesterol in control and STZ-induced diabetes rats has been reported in Table 3. A comparison graph between individual herbals and poly herbal extracts has been shown in Figure 1.

Figure 2. Antihyperglycemic activity of mixtures on STZ-induced diabetic rat

		Blood glucose level (mg/dL)				
Groups	**Dose**	**Zero Day (Fasting Blood Glucose Level)**	**1st Day (After STZ induction of Diabetes Blood Glucose**	**7th Day (After induction of diabetes with Treatment Blood**	**14th Day (After induction of diabetes with Treatment Blood**	**21st Day (After induction of diabetes with Treatment Blood Glucose**

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			Level)	**Glucose Level)**	**Glucose Level)**	**Level)**
Normal Control (NC)	Vehicle 2 ml/kg	75.83± 1.956	76.21± 0.598	75.26± 0.297	77.52± 0.264	75.22± 0.176
Diabetic Control (DC)	STZ (60 mg/kg)	76.5± 0.140	277.5± 0.201	281.2± 0.549	290.2± 0.524	315.2± 0.521
Alstonia scholaris Ethanol Extract (AS-EE)	200mg/kg	76.3± 0.166	283.8± 0.166	261.8± 0.133	219± 0.128***	151.67± 1.978 ***
Centella asiatica Ethanol Extract (CA-EE)	200mg/kg	75.52± 0.076	288.7± 0.044	259.8± 0.191	212± 0.166***	149.50± 1.717 ***
Corchorus trilocularis Ethanol Extract (CT- EE)	200mg/kg	76.12± 0.621	273.5± 0.181	256.4± 0.137	208.8± 0.319***	145.30± 1.870 ***
Morinda Pubescens Ethanol Extract(MP-EE)	200mg/kg	75.45± 0.141	285.12± 0.664	257.1± 0.166	201± 0.172***	132.00± 1.840 ***
Polyherbal Extract (PHE)	200mg/kg	75.59± 0.633	277.15± 0.241	253.8± 0.143	177± 0.186***	101.12± 0.121***
Glipizide(Standard)	5mg/kg	76.5± 0.140	273.5± 0.241	204.8± 0.172	112± 0.134***	79.83± 0.758*** ***

Data were expressed as Mean ± SEM (n=6) *P<0.05, ** P<0.01 and *** P<0.001 Vs Diabetic Control

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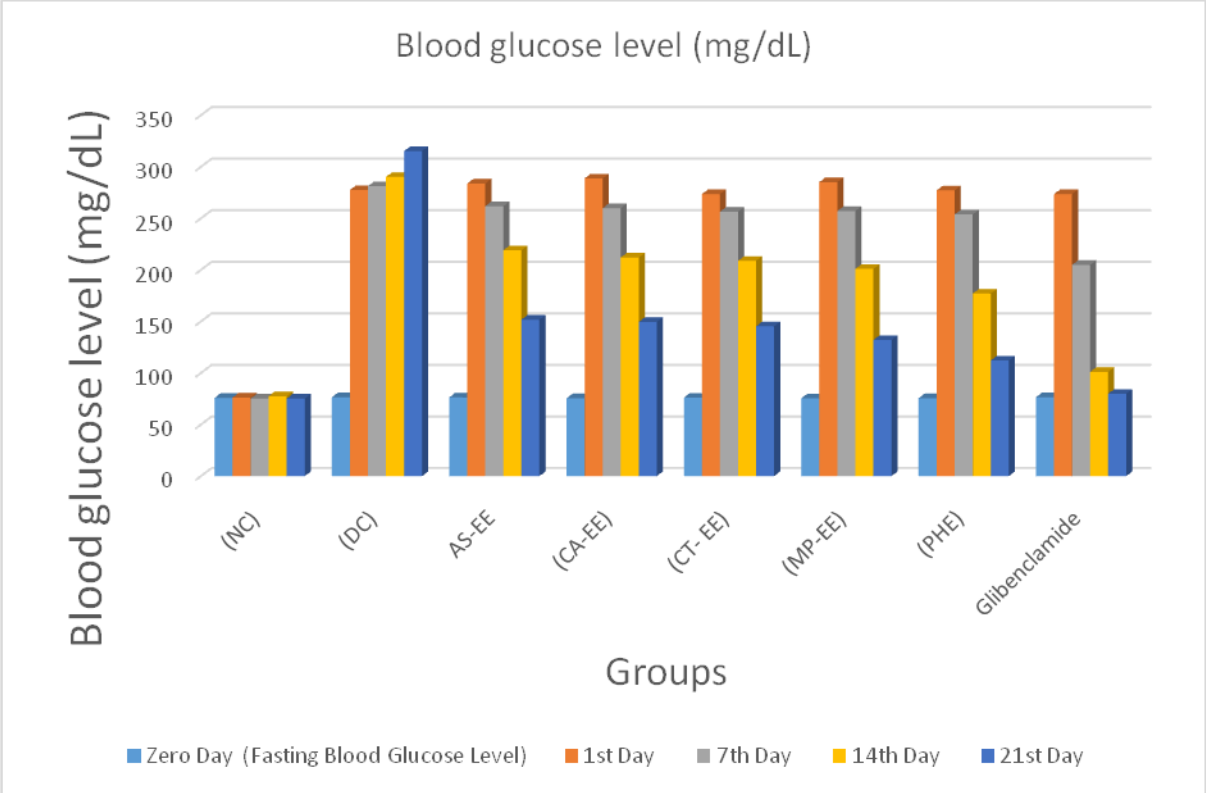

Fig 1.Comparative evaluation Blood Glucose level of extracts and Polyherbal extract

Figure 3. Effect on biochemical parameters in control and STZ-induced diabetic rats

S.No	Groups	SGPT (IU/dL)	SGOT (IU/dL)	Total protein (mg/dL)	Triglyceride (mg/dL)	Total cholesterol (mg/dL)
1	Normal control (2 mL/kg)	121.22±1.04	133.31±1.24	8.32±1.14	89.23 ± 2.31	140.3 ± 1.21
2	Diabetic control (2 mL/kg)	213.72±1.40	263.02±1.12	6.16±1.11	223.15 ± 1.10	263.13 ± 1.19
3	*Alstonia scholaris* Ethanol Extract (AS-EE)	139.16±1.09*	179.02±1.12*	7.03±1.59**	150.13 ± 1.12**	162.16 ± 1.13*
4	*Centella asiatica* Ethanol Extract (CA-EE)	142.29±1.31*	185.71±1.32*	7.19±1.24**	147.37 ± 1.11*	152.25 ± 1.17*
5	*Corchorus trilocularis* Ethanol Extract (CT- EE)	134.22±1.29**	172.02±1.73*	6.87±1.17*	156.3 ± 1.09*	153.06 ± 1.08*
6	*Morinda Pubescens* Ethanol Extract(MP-EE)	130.16±1.07**	178.17±1.52*	6.99±1.22*	144.3 ± 1.26*	150.21 ± 1.19*
7	Polyherbal Extract (PHE)	128.22±1.22**	142.64±1.21**	7.60±1.39**	111.23 ± 1.19**	147.11 ± 1.29**
8	StandardGlibenclamide(PC)	113.66±1.21***	131.09±1.33**	8.24±1.21***	103.64 ± 1.12***	134 ± 1.72***

Data were expressed as Mean ± SEM (n=6) *P<0.05, ** P<0.01 and *** P<0.001 Vs Diabetic Control

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Here we have evaluated the Synergestic hypoglycemic activity of the ethanolic extracts of *Alstonia scholaris*, *Centella asiatica*, *Corchorus trilocularis*and *Morinda Pubescens*and the marketed drug in streptozotocin-induced diabetic rat. From Figure 1 it can be observed that the polyherbal extract have maintained the blood glucose level around 100, which is a better result in comparison to individual herbals. As shown in Table 2, the Polyherbal extract significantly reduced the blood glucose level in STZ-induced diabetic rat.PHE showed significant antidiabetic activity at 21th days at 200 mg/kg dose levels. The diabetic control group exhibited significant increase ($p<0.001$) in blood glucose levels at all time periods in comparison to the normal control group. The polyherbal formulation indicated significant decrease ($p<0.01$) in blood glucose level at day 21 day against the diabetic control group and the reduction in blood glucose level was comparable to Glipizide at 21st day. All the plant extract have shown potential in their role to reduce the blood glucose level. Polyherbal extract showed synergestic activity as compared to the individual plants.The concept of polyherbal formulation is well documented in the ancient literature. Traditional medicine systems generally assume that synergy effects of all ingredients of the plants will bring about maximum therapeutic efficacy [32]. Herbal combinations are more effective than the constituent herb used alone [31]. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential, enhances the therapeutic action, and reduces the concentrations of single herbs, thereby reducing adverse events [25, 33]. It is believed that the synergistic interactions between the constituents are responsible for the therapeutic efficacy [33].Diabetes is associated with alternation of plasma lipid and lipoprotein and is consequently linked to increased risk of coronary heart disease. Insulin deficiency and increased blood glucose level lead to hyperglycemia and hypercholesterolemia, as was found in the diabetic control group in the present study. This is may be due to uninhibited actions of lipolytic hormones on the fat depots and increased mobilization of free fatty acids from the fat depot. This excess fatty acid is converted into phospholipids and cholesterol in liver. The elevated lipid levels were restored to near normal in the polyherbal extracts treated groups.The elevated levels of SGPT, SGOT, total protein, total cholesterol, and triglyceride in the diabetic control group reflected the significant alteration of liver function by STZ induction (Table 3). The extract was found to be equipotent to Glipizide in restoration of the elevated enzyme levels to normal, implying the normal functioning of liver. Several authors have reported that flavonoids, tannins, alkaloids, and terpenes are known to be bioactive antidiabetic principles . The antihyperglycemic effect of the extract is may be due in part to their flavonoids, alkaloids, tannins, and terpenes.

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*Alstonia scholaris*acts by increasing the production of insulin Leaves extract of *Alstonia scholaris* produced a highly significant decrease in blood glucose and a mechanism of this action was upon insulin triggering and direct insulin-like effects[14][34].Betulin and lupeol acetate were reported to exhibit hypoglycaemic activity[35].whereas the *Centella asiatica* [36,37] helps in regeneration and restoration of β cells of the pancreas. *Corchorus* trilocularisis reported to have insulin like action [38]. One study reports that *Morinda Pubescens*produces hypoglycemic activity due to PPAR γ/α agonist mechanism thus improving insulin resistance condition [22].Morinda may also have brought about hypoglycaemic action through stimulation of surviving ßcells of islets of Langerhans to release more insulin. This was clearly evidenced by the increased levels of plasma insulin in diabetic rats treated with Morinda[39].

CONCLUSION

The study indicates that the polyherbal extract at a dose of 200mg/kg body weight is showingeffective in significantly reducing blood glucose levels in diabetic rats and its anti-diabetic activity is comparable to Glipizide. The significant hypoglycemic activity of the polyherbal extract might be due to the varied mechanism of action of each of the herbal drug present in the formulation. Hence, the developed polyherbal formulation might prove to be a safe alternative for the existing anti-diabetic synthetic drugs. Further studies need to be carried out to explore the mechanism of action of each plant and to define the active phytochemicals present in each plant extract.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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