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Synergic Activity of Fenugreek seeds and Neem leaf extracts against Alloxan induced diabetic rats.

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Abstract: Diabetes is a metabolic disease in which a person has high blood sugar, either because the body does not produce enough insulin. Different studies have established the antidiabetic activity of fenugreek and neem. In present study our aim is to evaluate the antidiabetic effect of combined extracts in diabetic rats and find out the synergistic activity of both plant extracts if any. Glucose Tolerance Test suggested that efficacy of combined extract than their individual as the combined extract produced maximum fall of serum glucose levels 49.89% at 90 min after glucose administration. The Alloxan (120mg/kg .i.p.) used for induction of diabetes and Fenu Greek seed extract and Neem leaf extract used at a dose of 100mg/kg and 100mg/kg represents for single extract treatment, where as FSE and NLE 50mg/kg, 50mg/kg in combined extracts treatment for 28 days study. Glibenclamide used as standard drug. After 28 treatment the extracts significantly reduces serum glucose levels, the combined treatment found most effective. Combined extracts produces 52.45%, 20.45%, 24.58%,25.1%,24.6% reduction in serum cholesterol, triglycerides,LDLs, Creatinine.Urea respectively, increased 25.28% HDL levels after 28 days treatment which is better than the individual effect of extract. The results suggested the synergistic antidiabetic activity of MEFS and EENL when administered together.
Key words: Diabetes, glucose, Fenu Greek, Neem leaf Cholesterol, Urea, Creatinine, Triglycerides.

INTRODUCTION

Diabetes is a metabolic disease in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of Polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicines are important [1]. Plant drugs and formulations are frequently considered to be less toxic and more free side effects than synthetic one [2].There are numerous traditional medicinal plants reported to have hypoglycemic properties such as Allium sativum (Garlic), Azadirachta indica (Neem),

Vinca rosea (Nayantara), Trigonella foenum (Fenu Greek), Momordica charantia (Bitter ground), Ocinum santum (Tulsi). Many of these are less effective in lowering glucose levels in severe diabetes.

Fenugreek (*Trigonella foenum graecum*) is an annual herb that belongs to the family Leguminosae has definite anti hyperglycemic activity. Other pharmacological properties such as anti inflammatory, antioxidant, anti microbial, antiviral, hypotensive, and hypercholesterolemia are also exhibited [3]. The importance of Fenugreek is considered in the presence of metal contents which have a significant role in the biological activity.

Chromium a key constituent of the Glucose tolerance factor is vital to maintain proper blood sugar. Chromium supplementation can break the cycle of overeating sweets, at the
same time chromium breaks down glucose and fatty acids more efficiently within the body. Most of the herbs contain chromium but highest amount found in Fenugreek [4].

The active constituents involved in producing the above activities are pyridine type alkaloids like gentianine, trigonelline, choline, flavanoids-orientin, vitexin, quercetin; steroidal saponins –diosgenin, yamogenin, and gitogenin Several pharmacological activities and medicinal applications of various parts of neem are well known[5]. Biological activity of neem is reported with the crude extracts and their different fractions from leaf, bark, root, seed and oil[6]. However, crude extract of different parts of neem have been used as traditional medicine for the treatment of various diseases. Various parts of the neem tree have been used as traditional ayurvedic medicine in India from time immemorial. The medicinal utilities have been described, especially for leaf, fruit and bark. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident. Neem oil finds use to control various skin infections1. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis. However, apart from these uses, there are several reports on the biological activities and pharmacological actions of neem based on modern scientific investigations.[7]

In present study our aim is to evaluate the antidiabetic effect of combined extracts in diabetic rats and find out the synergistic activity of both plant extracts if any.

METHODS AND MATERIALS

Animals

Wistar rats of weight between 150 to 200 g obtained from NIN, Hyderabad, India, were used in the study. The animals were maintained under standard conditions in animal house of Vaageswari Institute of Pharmaceutical Sciences. The rats were males 8-10 weeks old with average weight of 150-200g. Animals were housed 3-4 per cage in a
Temperature-controlled (22±1) AC room, with a light/dark cycle of 12hr for a week following their arrival; the animals were allowed free access to the standard rat chow diet and tap water they were acclimating to the environment. Rats were also monitored daily and cages cleaned thrice weekly. At the start of the experiment animals were randomly distributed so that body weights, initial triglycerides (TG), total cholesterol (TC), other parameters in all the experimental groups were similar.

Collection of Plant material:

Fenugreek (Trigonella foenum) seeds were purchased from the local traders of karimnagar, Andhra Pradesh. And *Azadirachta indica* leaves are collected from Local areas of Karimnagar (dist), Andhra Pradesh, India.

Preparation of plant extract:

500gm of fenugreek seeds were obtained and washed. The collected seeds were dried at room temperature, pulverized by a mechanical grinder, sieved through 60 mesh and was macerated with 70% methanol for 5 days. The filtrate was dried, concentrated to dryness in vacuum and weighed [8] and the collected neem plant leaves was sun dried, Pulverized by a mechanical grinder, sieved through 40mesh. About 120g of powdered materials were extracted using ethanol (50°C) using soxhalate apparatus[9]. The extraction was carried out until the extractive becomes colorless. The extracts is then concentrated by distillation process and dried under reduced pressure. The solvent free semisolid mass thus obtained is used for the experiment. This semisolid mass contains the active compound Nimbi din [10].

EXTRACT AND DRUG ADMINISTRATION

Before use, the extracts and standard drug glibenclamide were reconstituted in Normal saline (vehicle) and administered orally via gastric intubation. Methanolic extract of Fenu greek seeds (MEFS) and Ethanolic extract Neem leaves(EENL) used at a dose of 100 mg/kg and 100 mg/kg *b.w.* respectively for single. Extract treatment, where as

MEFS (50 mg/kg) and EENL (50 mg/kg) in combined extracts treatment.
Glibenclamide was administered at a dose of 0.5 mg/kg. The dosage of the extracts was determined from different literature mentioned above. The controls received normal Saline.

Oral glucose tolerance test (OGTT)
The oral glucose tolerance test was performed in overnight fasted normal animals.Rats divided into five groups each consists six animals. The different groups were administered with normal saline, glibenclamide, FENU GREEK SEEDS extract, *NEEM* leaves extract, both extract respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation at 0, 30, 60, 90 and 120 min of extract administration and blood glucose levels were estimated[11].

Induction of experimental diabetes
Animals were fasted overnight and diabetes was induced by the intraperitoneal Injection of alloxan monohydrate in normal saline at a dose of 120 mg/kg body weight.

The animals were kept under observation and the fasting blood glucose level was determined after 48 h of alloxan injection. The animals showing hyperglycemia were used for the study [12].

Experimental design

Alloxan-induced diabetic rats (mentioned above) were divided into five groups each consists six animals, and normal mice were used as the control.

Group 1: Normal control, normal rats were treated with normal saline.

Group 2: Diabetic control, diabetic mice treated with normal saline.

Group 3: *fenugreek extract* treated group, diabetic mice treated with 100 mg/kg of MEFS.

Group 4: *neem extract* treated group, diabetic mice treated with 100 mg/kg of EENL.

Group 5 Standard drug treated group, diabetic mice treated with 0.5 mg/kg glibenclamide.

Group 6: Combined extract treated group, diabetic mice treated with 50 mg/kg of MEFS and 50 mg/kg EENL.

All groups were administered orally by gastric Incubation once a day. The blood samples were drawn on 0^{th}, 7th, 14th, 21st and 28th day from the retro orbital venous plexus of rats under ether anesthesia and the blood was Centrifuged at 2,500 rpm for 10 min.
The serum thus obtained was used for biochemical estimation of blood glucose, total Cholesterol (CHL), triglycerides (TG), high density lipoprotein cholesterol (HDL) and Low density lipoprotein cholesterol (LDL)[12].

Bodyweight determination
Literature and case study showed that body weight decreases in diabetic patient.

Therefore, mean body weight of different group of animals was measured during treatment on weekly basis.

Biochemical parameters
Blood glucose, CHL, TG, HDL and LDL kit were obtained from Excel Diagnostics Pvt. Ltd. Blood glucose, CHL, TG, HDL and LDL levels in serum were measured using a semi-auto analyzer as per methods described by the manufacturer.

Statistical analysis
The results were expressed as MEAN±SEM. Statistical analysis was performed by One-way analysis of variance (ANOVA) test for multiple comparisons followed by Turkey-Kramer test. The values were considered significant when $P< 0.05$.

Effect of combination treatment on glucose tolerance test
Table 1 depicts the hypoglycemic effects of single oral administration of the Extracts individually (MEFS - 100 mg/kg and EENL - 100 mg/kg) and in combination (MEFS - 25 mg/kg + EENL - 175 mg/kg). Individual extract treated group increased the Glucose levels were seen after 30 min and hypoglycemia effect was observed only at 90 Min, whereas rats treated with standard drug and combination of both *Fenugreek seed Extract* and *Neem leaf* extracts showed hypoglycemic activity at 60 min. The Combination treatment produced a maximum fall of 53.71% at 90 min after glucose administration.

Effect of MEFS and EENL on fasting serum glucose level

In order to investigate synergistic antidiabetic activity of *Fenugreek extract* and *Neem leaf extract*, MEFS and EENL were given individually and in combination. **Table 2** describes the effect of treatment of the extracts on serum glucose levels. Treatment with Standard drug, extract alone and in combination decreases serum glucose level Significantly. After 28 days treatment fasting serum glucose level of combined extract treated animals were found 81.3 mg/dl, where as in METT, MEAS and glibenclamide treated group fasting serum glucose level were 95.0, 94.2 and 85.6 respectively. Results suggested synergetic antidiabetic potential of combination treatment.

Effect of individual and combination extracts on body weight

Diabetic rats showed a significant decrease in body weight was observed on 7th, 14th, 21st and 28th day, when compared to the normal group. Treatment with glibenclamide and extracts reverse the trend which is observed by the increase in body weight. At the end of 28 days treatment the body weight of glibenclamide, MEFS,EENL and combination extract treated group, increased significantly by 7.9, 4.4, 4.1 and 9.2% respectively. The combination treatment produces better effect than the individual treatment (Table 3).

Effect of MEFS and EENL alone and in combination on serum lipid profile

Table 4-9 describes the effect of extracts on serum lipid profile. An increase in the serum triglycerides, total cholesterol and LDL levels, Urea,Creatinine and an increase in the HDL cholesterol levels were observed in diabetic rats.

When compared to the diabetic control rats, significant reductions of CHL were observed in drug treated group. Treatment with MEFS, EENL produced 37.1 and 35.8% reduction in CHL after 28 days, and 56.1% reduction in CHL level were found in combination extract treated group (Table 4).

Treatment with MEFS, EENL produced 18.3 and 16.78% reduction in Triglycerides after 28 days, and 20.45% reduction in TG level were found in combination extract treated group (Table 5).

Treatment with MEFS, EENL produced 20.12 and 22.24% increased in HDL after 28 days, and 25.38% increased in HDL level were found in combination extract treated group (Table 6).

Treatment with MEFS, EENL produced 19.03 and 21.13% reduction in LDL after 28 days, and 24.58% reduction in LDL level were found in combination extract treated group (Table 7).

Treatment with MEFS, EENL produced 21.24 and 22.25% reduction in Creatinine after 28 days, and 25.1% reduction in Creatinine level were found in combination extract treated group (Table 8).

Treatment with MEFS, EENL produced 20.34 and 22.38% reduction in Urea after 28 days, and 24.6% reduction in Urea level were found in combination extract treated group (Table 9).

Results were proving the synergetic antihyperlipidemic efficacy of MEFS and EENL when extract were administered in combination.

TABLE 1: Effect of Fenugreek seeds extract and Neem leaf extract alone and in combination on the serum glucose levels in glucose loaded rats

GROUPS	DRUG	0min	30min	60min	90min	120min
I	Normal control	113.2±7.43	113.2±7.43	114.4± 8.84	111± 9.97	111.4± 7.73
II	Diabetic Control	179.4±17.3	351.8±91.259	437.6±88.71	485± 43.92	474.6± 37.44
III	Diabetic Control + MEFS(100mg/kg)	287.2 ±13.2	280 ±18.5	262.6 ±20.8	238.8 ±24.5	190.8 ±29.4
IV	Diabetic Control + EENL(100mg/kg)	229±16.7	199±17.46	178±11.51	136.5±14.96	122.24± 59.61
V	Diabetic Control + Glibenclamide	289.6 ±3.8	282.8 ±4.9	272 ±6.51	240.2 ±.96	191.8 ±5.4
VI	MEFS,50mg/kg +EENL,50mg/kg	272.2 ±10.2	265 ±15.72	251.6 ±14.8	223.8 ±20,13	172.8 ±12.72

TABLE 2: Effects on serum glucose level after the administration of MEFS and EENL alone and in combination in alloxan-induced diabetic rats

GROUPS	DRUG	0 th day	7th day	14th day	21st day	28th day
I	Normal control	113.2±7.43	100.1±7.43	99.53± 8.86	95.76± 2.01	90.1± 5.23
II	Diabetic Control	179.4±17.3	335±1.2	350.01±2.23	355.1± 4.80	358.12± 9.89
III	Diabetic Control + MEFS(100mg/kg)	287.2 ±13.2	203 ±10.1	146.98 ±2.78	124.98±24.5	103.61 ±22.1
IV	Diabetic Control + EENL(100mg/kg)	223±12.8	194±15.12	131.12±11.51	120.96± 12.16	110.86± 12.54
V	Diabetic Control + Glibenclamide	289.6 ±3.8	250.2 ±4.6	180.5 ±1.23	130.3 ±.86	95.3 ±2.12
VI	MEFS,50mg/kg +EENL,50mg/kg	272.2 ±10.2	180.23±19.1	123.35 ±13.9	110.45±10.56	94.65 ±1.23

TABLE 3: Effect of extracts alone and in combination on the body weight (gm)

GROUPS	DRUG	0 th day	7th day	14th day	21st day	28th day
I	Normal control	113.2±7.43	100.1±7.43	99.53± 8.86	95.76± 2.01	90.1± 5.23
II	Diabetic Control	179.4±17.3	335±1.2	350.01±2.23	355.1± 4.80	358.12± 9.89
III	Diabetic Control + MEFS(100mg/kg)	287.2 ±13.2	203 ±10.1	146.98 ±2.78	124.98±24.5	103.61 ±22.1
IV	Diabetic Control + EENL(100mg/kg)	223±12.8	194±15.12	131.12±11.51	120.96± 12.16	110.86± 12.54
V	Diabetic Control + Glibenclamide	289.6 ±3.8	250.2 ±4.6	180.5 ±1.23	130.3 ±.86	95.3 ±2.12
VI	MEFS,50mg/kg +EENL,50mg/kg	272.2 ±10.2	180.23±19.1	123.35±13.9	110.45±10.56	94.65 ±1.23

TABLE 4: Effect of MEFS and EENL on serum total cholesterol (mg/dl)

GROUPS	DRUG	0 th day	7th day	14th day	21st day	28th day
I	Normal control	70.38± 1.34	71.38± 0.84	71.34± 1.66	71.5± 0.6	70.5±1.34
II	Diabetic Control	288.2± 1.31	285.63± 1.81	286.63± 1.49	286.53± 2.06	285.4±2.06
III	Diabetic Control + MEFS(100mg/kg)	301±3.5	245±4.3	192.2 ±4.8	143±4.0	125.4±4.91
IV	Diabetic Control + EENL(100mg/kg)	271.95± 2	230.2± 1.42	198.87± 0.97	156.8±0.90	111.34±1.3
V	Diabetic Control + Glibenclamide	299.76±3.2	228.0±3	174.1±3	125.1 ±3.4	116.89±2.5
VI	MEFS,50mg/kg +EENL,50mg/kg	245.15± .86	212.13± 3.34	185.12± 9.12	136.23± 2.24	104.12±3.5

TABLE 5: Effect of MEFS, EENL alone and in combination on serum triglycerides(mg/dl)

GROUPS	DRUG	0 th day	7th day	14th day	21stday	28th day
I	Normal control	87.32± 5.4	87.62± 5.2	87.78± 4.9	88.08± 5	87.6±4.5
II	Diabetic Control	170.3± 2.1	170.30± 3.6	170± 2.7	169.6± 2.5	170.1±2.3
III	Diabetic Control+ MEFS(100mg/kg)	178.9 ±4.5	157.18±4.2	149.4±4.4	145.6 ±4.2	133.48±4.3
IV	Diabetic Control+ EENL(100mg/kg)	132.15± 2.1	123.97± 1.9	116.6± 2.4	108.65±2.79	95.6±3.4
V	Diabetic Control+ Glibenclamide	131.5± 3.2	125.35± 4.2	110.25±4.05	100.47± 2.3	94.3±3.4
VI	MEFS,50mg/kg+ EENL,50mg/kg	130.23± 1.23	121.12±1.56	112.34± .23	104.24±3.24	94.35±2.4

TABLE 6: Effect on serum HDL cholesterol level after administration of MFS, EENL alone and in combination (mg/dl)

GROUPS	DRUG	0 th day	7th day	14th day	21st day	28th day
I	Normal control	52.12± 2.23	46.23± 3.68	48.34± 2.86	48.35± 5.08	50.65±9.43
II	Diabetic Control	45.3± 2.6	41.30± 8.6	39± 2.8	36.6± 2.9	30.1±2.8
III	Diabetic Control+ MEFS(100mg/kg)	50.9 ±4.9	52.24±2.32	54.64 ±4.9	58.32 ±4.9	62.44±4.6
IV	Diabetic Control+ EENL(100mg/kg)	43.15± 2.13	48.34± 1.23	50.6± 2.42	51.66± 2.34	60.12±4.56
V	Diabetic Control+ Glibenclamide	51.5± 3.2	51.54± 4.21	54.25± 8.43	58.49± 2.43	65.2±2.65
VI	MEFS,50mg/kg+ EENL,50mg/kg	50.23± 3.23	54.12± 8.39	58.32± 1.28	64.28± 9.42	65.23±1.23

TABLE 7: Effect on serum LDL level after administration of MEFS, EENL alone and in combination (mg/dl)

GROUPS	DRUG	0 th day	7th day	14th day	21st day	28th day
I	Normal control	42.19± 1.26	44.23± 3.69	49.34± 2.88	51.36± 8.45	54.68±8.46
II	Diabetic Control	100.3± 2.9	101.24±8.65	112± 3.76	116.43± 2.92	117.86±1.25
III	Diabetic Control + MEFS(100mg/kg)	98.54 ±2.34	92.24±2.38	90.64±4.94	85.78 ±8.65	82.40±5.43
IV	Diabetic Control + EENL(100mg/kg)	85.45± 1.24	78.64± 1.33	76.6± 9.65	70.08± 1.24	65.66±5.43
V	Diabetic Control + Glibenclamide	81.5± 3.8	78.54± 4.28	74.29± 8.43	68.42± 9.08	66.78±2.89
VI	MEFS,50mg/kg +EENL,50mg/kg	80.23± 5.65	74.82± 6.73	68.38± 9.12	62.48± 9.55	60.28±5.46

TABLE 8: Effect on serum creatinine level after administration of MEFS, EENL alone and in combination (mg/dl).

GROUPS	DRUG	0 th day	7th day	14th day	21st day	28th day
I	Normal control	0.386 ±0.02	0.384 ±0.02	0.374±0.02	0.368 ±0.01	0.378±0.02
II	Diabetic Control	6.22 ±0.31	6.38 ±0.32	6.26 ±0.30	6.32 ±0.33	6.34 ±0.3
III	Diabetic Control + MEFS(100mg/kg)	6.34 ±0.35	5.32 ±0.17	4.94 ±0.19	3.64 ±0.27	2.24 ±0.24
IV	Diabetic Control + EENL(100mg/kg)	2.37± 0.17	2.02± 0.17	1.8±0.16	1.62± 0.17	1.5±0.12
V	Diabetic Control + Glibenclamide	6.32 ±0.15	5.18±0.17	3.74 ±0.02	2.94 ±0.20	2.12 ±0.21
VI	MEFS,50mg/kg +EENL,50mg/kg	6.14± 4.54	5.32± 4.32	3.38± 1.2	1.43± 2.33	1.21±2.24

TABLE 9: Effect on serum urea level after administration of MEFS, EENL alone and in combination (mg/dl).

GROUPS	DRUG	0 th day	7th day	14th day	21st day	28th day
I	Normal control	29.1± 1	28.4±1.6	28.6 ±6.89	28.9±1.4	29.4±1.4
II	Diabetic Control	147.1 ± 1.5	145.6±3.22	145.3 ±1.9	146.8±2.6	144.6±2.2
III	Diabetic Control+ MEFS(100mg/kg)	146.1 ±4.9	141.94±5.2	134.5 ±4.9	125.1±7.4	82.8±6.8
IV	Diabetic Control+ EENL(100mg/kg)	92.45± 2	90.3± 2.9	87.42± 3.6	80.57± 2.8	74.37±2
V	Diabetic Control+ Glibenclamide	148±8.6	134.4±9.8	123.5 ±12.9	117.3±12.6	72.1± 12.44
VI	MEFS,50mg/kg+EENL,50mg/kg	90.32± 2.35	85.76± 2.65	80.45± 1.24	70.86± 1.98	69.86±4.54

DISCUSSION

FENU GREEK SEED EXTRACT and *NEEM LEAF EXTRACT* were investigated against alloxan induced diabetic rats. Induction of alloxan causes destruction of pancreatic beta cells in a wide variety of animal species. Depending upon species and amount of alloxan it damages a large number of beta-cells and causes decrease in endogenous insulin secretion.

Acute administration of sulfonyl urea increases insulin release from the pancreas, since in Our study the glibenclamide reduced blood glucose levels in diabetic rats, so the state of diabetes is not severe or alloxan caused less number of beta cell destruction. Increase in concentration of CHL, TG, LDL, Creatinine.Urea and decreased HDL is observed in alloxan induced untreated diabetic rats. Hyperlipidemia is a recognized complication of diabetes mellitus.

The *FENU GREEK SEED EXTRACT* and *NEEM LEAF EXTRACT* administration almost reversed these effects as it reduced total CHL, TG, LDL,Creatinine,Urea concentration and increased HDL notably in combination. In this context, combination of *FENU GREEK SEED EXTRACT a* and *NEEM LEAF EXTRACT* was found to be as effective as glibenclamide in reducing the plasma lipid profiles in diabetic rats. HDL act Via transporting cholesterol from peripheral tissues to the liver and protect damage of membrane and reduces chance of coronary disease. Studies showed that isolated alkaloids are responsible for ant diabetic activity of *FENU GREEK SEED EXTRACT* and Several alkaloids are isolated from the leaves of *NEEM* which may responsible for its antidiabetic activity. Though in combination both the extracts given in less dose than their individual dose but the combination produces superior effect than the individuals.

CONCLUSION

The results of the study reveal the synergestic activity of *Fenu Greek seed extract* and *Neem leaf extract* in combination against alloxan induced diabetes and hyperlipidemia in Rats. The combination of these two drugs may confer prominent antidiabetic effect in future.

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