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Research Article

HYPOGLYCEMIC AND ANTIDIABETIC POTENTIAL OF CHITOSAN AQUEOUS EXTRACT OF *ELAEOCARPUS GANITRUS*

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ABSTRACT

In the information regarding the use of *Elaeocarpus ganitrus* leaves for several disorders systemic studies were carried out in our laboratory to establish the antidiabetic potential of chitosan based extract of *Elaeocarpus ganitrus* and antidiabetic activity of aqueous extract of *Elaeocarpus ganitrus*. In the present study rats were used as experimental animals. It was found that chitosan based leaf extract of *Elaeocarpus ganitrus* produced hypoglycaemic effect in normal rats. The study indicates clinically significant antidiabetic activity of *Elaeocarpus ganitrus* in diabetic rats. The chitosan based extract improved the antidiabetic activity of *Elaeocarpus ganitrus* clearly indicating synergism.

Keywords: *Elaeocarpus ganitrus,* Chitosan, Antidiabetic potential.

INTRODUCTION

Elaeocarpus ganitrus is a medium sized tree[1]. It is used in decreasing the heat in the body. It is supposed to be a good agent in treating mental abnormalities. It is a good pain relieving agent and used as antioxidant[2]. It is very helpful in improving peristaltic movements in the intestine and also it is helpful in stimulating liver for better functioning. It is supportive in producing the bile secretion. It also helps in maintaining blood density. It also helps in relieving spasm from the body especially on the respiratory tract. It also helps in maintaining the proper body temperature. Chitosan is a natural product which is derived from the polysaccharide chitin[3]. Chitin is an aminopolysaccharide, abundantly available as natural biopolymer found in the exoskeletons of crustacean like shrimp, crabs, lobster and other shellfish. Partial deacetylation of chitin to remove acetyl groups present in chitin gives chitosan[4]. Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4) linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit).

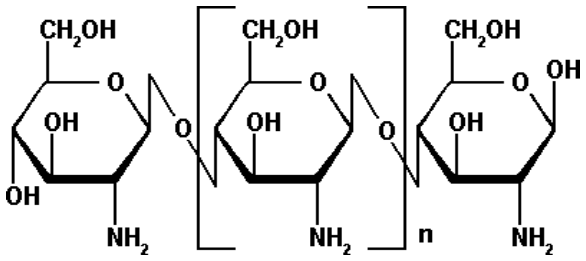

Fig. 1: Structure of Chitosan

It is generally accepted that soluble dietary fibers increase gastrointestinal lumen viscosity and delay gastric emptying[5]. Chitosans have specifically been shown to alter bile acid composition, increase neutral sterol excretion and reduce fat digestibility. The mechanisms by which chitosans achieve these effects are increased intestinal viscosity and increased bile acid-binding capacity. Chitosan has such characteristics that are associated with a dietary fiber which are assumed to be related to the reductions in cholesterol as well as increases in the excretion of neutral steroids

observed in animal experiments. Chitosan, which is largely deacetylated, contains cationic groups located on the polyglucosamine chain. Thus, chitosan may have a bile acid-binding capacity, causing entrapment or disintegration of mixed micelles in the duodenum and ileum. This interruption in bile acid circulation would lead to reduced lipid absorption and increased sterol excretion. Chitosan is relatively insoluble in water but is soluble in dilute acids, giving rise to highly-viscous dietary fibers. It has been suggested that viscous dietary fibers such as chitosan inhibit uptake of dietary lipids by increasing the thickness of the intestinal lumen boundary layer, a proposal again supported by numerous animal experiments. Streptozotocin or Streptozocin or Izostazin or Zanosar (STZ) is a synthetic antineoplastic agent that is an anti-tumour antibiotic[6] and chemically is related to other nitrosureas used in cancer chemotherapy. It is a nitromethylurea[7] derivative of 2-deoxy glucose and alkylating agent similar in reactivity to the other nitrosomethylureas, except that its glucose moiety causes it to be especially taken up in the pancreas.

EXPERMENTAL

Materials

Glucose kit constituents the following reagents

Glucose reagent 1 : Glucose oxidase, Peroxidase, 4- amino antipyrine, Hydroxy benzoate.

Glucose diluents : Phosphate buffer, pH 7.4, Phenol.

Glucose standard : Dextrose (100mg/dL).

Working reagent preparation

The contents of 1 vial of Glucose reagent-1 were transferred quantitatively to a clean black colored plastic bottle provided in the kit. The bottle was reconstituted with 50 ml of glucose diluents.

Storage of working reagent

The working reagent was stable for 12 months from the date of reconstitution when stored at 2-8 ^{0}C.

Estimation of blood glucose in rats

In this study the enzymatic glucose oxidase-peroxidase (GOD/POD) method[8] was used.

Glucose oxidase-peroxidase (GOD/POD) method

Glucose kit based on Trinder's method in which glucose oxidase (GOD) and peroxidase (POD) enzymes were used along with the chromogen 4-aminoantipyrine and phenol. This method is one step, simple and rapid. Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalyzed reaction the oxygen liberated is accepted by the chromogen system to give a red colored quinoneimine compound. The red colour quinoneimine dye so developed is measured at 505 nm using autoanalyzer (Screen master 3000, Tulip).

$$\text{Glucose} + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{P-hydroxybenzoate} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye}$$

Specimen collection

The blood was left in the sample collection tubes without adding any anticoagulant. Then centrifuged at 3000 rpm for 10 min. The serum was separated and used for the analysis. Serum should be separated within 30 minutes, as the rate of glycolysis in blood is approximately 7 mg/hour at room temperature. Serum glucose is stable for 8hr at room temperature and for 72 hr at 2-8^{0}C.

Equipment: Screen Master 3000 (Tulips)

Programme: The basic assay parameters were

Mode	: End point
Wavelength	: 505 nm (490-550 nm)
Temperature	: 37^{0}C
Optical path length	: 1 cm
Blank	: Reagent blank
Incubation	: 10 min at 37^{0}C
Sample volume	: 10 µl
Working reagent volume	: 1 ml
Concentration of standard	: 100 mg/dL
Linearity	: up to 500 mg/dL
Stability of color	: 1 hour
Units	: mg/dL

PROCEDURE

Wavelength/ filter : 505nm (Hg546 nm)/ Green
Temperature : 37^0C/ R.T.
Light path : 1cm

Pipette the amount specified into clean dry test tubes labeled as Blank (B), Standard (S), and Test (T):

Addition sequence	B (ml)	S (ml)	T (ml)
Glucose reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Glucose standard (S)	-	0.01	-
Sample	-	-	0.01

Calculation

The percentage of blood glucose reduction was calculated by using the following equation.

% blood glucose reduction = (Xdi – Xdf / Xdi) x 100

Xdi = blood glucose level at zero hour.
Xdf = blood glucose level at that time.

Note: Unused working glucose reagent should be refrigerated immediately.

Standard glucose solution: Available as ready for use solution (100 mg/dl) in the glucose kit.

Studies on the hypoglycemic & Antidiabetic potential of Chitosan based extract of *Elaeocarpus ganitrus*

Diabetes mellitus refers to a group of disorders characterised by absent or deficient insulin secretion or peripheral insulin resistance, resulting in hyperglycaemia and impaired glucose metabolism and to know at what extent the leaf extract of *Elaeocarpus ganitrus* can correct the abnormality. To know the hypoglycaemic effect of chitosan based extract of *Elaeocarpus ganitrus* in normal rats on acute treatment the following parameter namely blood glucose was estimated before and after treatment with different doses. To know the antidiabetic potential of chitosan based extract of *Elaeocarpus ganitrus* in streptozotocin induced diabetic rats, on acute treatment the following parameter namely blood glucose was estimated after treatment with different doses in streptozotocin induced diabetic rats.

Preparation of the extract

Collection of material

The leaves of *Elaeocarpus ganitrus* were collected from the Forest Development Office, Addathegala, East Godavari Dt., A.P.

Preparation of aqueous extracts of *Elaeocarpus ganitrus*

The leaves were dried under shade dry for 7 days and grind them to get fine powder. Then the powder was used for the preparation of extract. 100gms of the leaf powder was taken into the beaker and make slurry with distilled water and kept aside for 24hrs. Then filtered it by musclien cloth and the extract was weight calculated was 8.3gms.

Preparation of chitosan based leaf extract of *Elaeocarpus ganitrus*

1gm of chitosan mixed in Tween80 and 100gm of the leaf powder taken in to the beaker and makes slurry with distilled water and kept aside for 24hours. Then filtered it by musclien cloth and the extract weight calculated was 7.3gms.

Preparation of drug solutions

As leaf extract of *Elaeocarpus ganitrus* and chitosan based extract of *Elaeocarpus ganitrus* are water soluble the drug solutions are prepared by dissolving the extracts in distilled water. As chitosan and glimepride are insoluble in water, they were soluble in Tween80 and forms suspensions.

Oral administration of drugs

For the administration of drugs, a 16 gauge hypodermic oral feeding needle purchased from local market was used. The tip of the needle was covered with suitable polyethylene tube of 1 cm length. The animal was grasped securely by the nap of the neck holding whole animal with left hand. The needle was attached to a syringe introduced through intradental space gently into the oesophagus and then the drug solution was introduced slowly by pushing the syringe (Ghosh MN, 1971) and the needle was withdrawn.

Acute studies in normal rats

Materials: *Elaeocarpus ganitrus* leaf extract
Albino rats (Mahaveer Enterprises, Hyderabad).
Drug : Glimepride and Chitosan.

Normal rats of either sex weighing 200-300 gm were used in the study. Animals were divided into five groups of six each and were provided with standard Gold Mohars pellet diet and water *ad libitum*. Animals were fasted about 16 to 18 hours prior to experiment, giving access to water. Groups II, III, IV & V were given orally the extracts of *Elaeocarpus ganitrus* & chitosan. Group I served as control.

Methodology

Group I: Administered vehicle serve as control.
Group II: Administered aqueous extract of *Elaeocarpus ganitrus* 100 mg/kg.
Group III: Administered chitosan based extract of *Elaeocarpus ganitrus* 100mg/kg.
Group IV: Administered chitosan based extract of *Elaeocarpus ganitrus* 200 mg/kg.
Group V: Administered chitosan 10 mg/kg.
The blood samples were collected into the Eppendroff's centrifuge tubes (1.5 ml, Tarson) at 0, 1st, 4th and 7th day intervals from all the groups of rats after the extract administration. Every time about 0.2 ml of blood was collected. The serum was separated by centrifuging the samples and the serum samples (0.1ml) was transferred using automated pipette and was analyzed immediately for blood glucose by GOD/POD method.

Acute studies conducted in diabetic rats

Induction of diabetes

Materials: Streptozotocin, Sodium chloride and Albino rats.
Animals were allowed to fast 24h and were injected with freshly prepared normal saline solution of streptozotocin at a dose of 65mg/kg body weight through intraperitoneal route. The serum glucose levels were measured after 20h. The serum glucose levels were observed up to 7days. Rats showing serum glucose level around 200-350 mg/dL were selected for the study.
Diabetic rats of either sex 170-200gm were used in the study. Animals were provided with standard diet (Rayan's Biotechnologies Pvt. Ltd, Hyderabad, India) and water *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethical Committee and by the regulatory body of the government. They were fasted for about 16-18h prior to collection of blood samples providing access to water. Total diabetic rats having blood glucose level 200-350mg/dL were divided into six groups of six each.

Materials

Glimepiride : Dr. Reddy's Laboratories, Hyderabad.
Chitosan : Sribionic Labs, Nellore.
Glucose kits : Choral biosystems, Crest groups, Tadepalligudem.
Albino rats : Mahaveer Enterprises, Hyderabad.
Plant leaves : Forest development office, Addathegala.

Methodology

The rats were divided into eight groups consisting of six rats each.
Group I : Administered vehicle serve as control.
Group II : Administered streptozotocin (65 mg/kg I.P.) serve as diabetic control.
Group III : Administered tween80 to the diabetic rats serve as vehicle control.
Group IV : Diabetic rats treated with Aqueous extract of *Elaeocarpus ganitrus* 100 mg/kg.
Grop V : Diabetic rats treated with Chitosan based extract of *Elaeocarpus ganitrus* 100mg/kg.
Group VI : Diabetic rats treated with Chitosan based extract of *Elaeocarpus ganitrus* 200mg/kg.
Group VII : Diabetic rats treated with Chitosan 10mg/kg.
Group VIII : Administered reference Standard Glimepiride 20mg/kg.

Collection of blood samples

The blood samples were withdrawn on 0, 1st, 4th & 7th day from the retro orbital plexus of rats. A fine glass capillary was inserted gently in the inner angle of the eye, then the capillary slides under the eye ball at 45^0 angles and over the bony socket to rupture the fragile venous capillary of the ophthalmic venous plexus. The passage was about 10 mm. The tip of the capillary was slightly retracted and the blood collected in the orbital cavity flows out from the capillary, which was collected in a tube. Capillary tube should be held gently, merely resting on fingers while blood was flowing. After collecting the desired volume, capillary was removed with simultaneous release of pressure by forefinger and thumb. Any residual blood droplet around the eyeball was wiped off with dry cotton wool. The blood samples were collected into the Eppendroff's centrifuge tubes (1.5 ml, Tarson) at 0, 1st, 4thand 7th day intervals from all the groups of rats after the extract administration. Every time about 0.2 ml of blood was collected. The serum was separated by centrifuging the samples and the serum samples (0.1ml) was transferred using automated pipette and was analyzed immediately for blood glucose by GOD/POD method.

RESULTS

Results of hypoglycemic activity on normal rats

Control group data is given in Table 1

Table 1: The blood glucose levels of normal rats (control, n=6) expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	79	74	80	92
R2	74	77	84	97
R3	89	82	87	91
R4	78	84	98	104
R5	78	87	89	105
R6	84	76	87	97
MEAN ± SEM	80.33 ± 1.97	80 ± 1.89	87.5 ± 2.24	97.6 ± 2.17

The blood glucose levels before and after the treatment with *Elaeocarpus ganitrus* leaf extract and chitosan based extract of *Elaeocarpus ganitrus* in normal and diabetic rats are given in Tables 2, 3, 4 and 5.

Table 2: The blood glucose levels of Chitosan (10mg/kg body weight, oral) on normal rats (control, n=6) expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	97	87	78	75
R2	96	85	76	76
R3	104	98	87	79
R4	98	93	85	69
R5	105	96	88	78
R6	98	86	79	78
MEAN ± SEM	99.66 ± 1.42	90.83 ± 2.06	82.16 ± 1.90	75.83 ± 1.35

In normal rats chitosan at the dose level of 10mg/kg body weight produces maximum blood glucose reduction of 8.86%, 17.55%, 23.91% respectively at 1, 4 & 7 days after administration.

Table 3: The blood glucose levels of aqueous extract of *Elaeocarpus Ganitrus* (100mg/Kg body weight, oral) on normal rats (control, n=6) expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	97	84	80	72
R2	87	79	64	67
R3	109	92	87	84
R4	104	94	88	74
R5	95	81	71	69
R6	94	96	87	77
MEAN ± SEM	97.66 ± 2.90	87.66 ± 2.68	79.5 ± 3.71	73.83 ± 2.27

In normal rats aqueous extract of *Elaeocarpus ganitrus* at the dose level of 100 mg/kg body weight produces maximum blood glucose reduction of 10.17%, 18.53%, 24.40% respectively at 1, 4 & 7 days after administration.

Table 4: The blood glucose levels of chitosan based aqueous extract of *Elaeocarpus ganitrus* (100mg/kg body weight, oral) on normal rats (control, n=6) expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	98	85	67	57
R2	104	88	74	64
R3	97	87	65	63
R4	103	87	84	72
R5	98	88	72	69
R6	89	77	74	65
MEAN ± SEM	98.16 ± 1.98	85.3 ± 1.57	72.6 ± 2.48	65 ± 1.92

In normal rats chitosan based leaf extract of *Elaeocarpus ganitrus* at the dose level of 100mg/kg body weight produces maximum blood glucose reduction of 13.10%, 26.03%, 33.78% respectively at 1, 4 & 7 days after administration.

Table 5: The blood glucose levels of chitosan based aqueous extract of *Elaeocarpus ganitrus* (200mg/kg body weight, oral) on normal rats (control, n=6) expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	98	75	67	47
R2	104	88	67	57
R3	97	84	67	53
R4	107	87	74	62
R5	97	87	72	66
R6	89	76	73	64
MEAN ± SEM	98.66 ± 2.32	82.83 ± 2.17	70 ± 1.24	58.16 ± 2.51

In normal rats chitosan based leaf extract of *Elaeocarpus ganitrus* at the dose level of 200mg/kg body weight produces maximum blood glucose reduction of 16.55%, 29.04%, 41.05% respectively at 1, 4 & 7 days after administration.

The percentage blood glucose reduction in normal and diabetic rats and their control values are given in Table 6.

Table 6: Comparison of hypoglycemic effect of chitosan based leaf extract of *Elaeocarpus ganitrus* with control in normal rats

S. No.	0 day		1st day		4th day		7th day	
	%change in blood glucose	Statistical significance p<0.05	%change in blood glucose	Statistical significance p<0.05	%change in blood glucose	Statistical significance p<0.05	%change in blood glucose	Statistical significance p<0.05
Chitosan	0.00	No	8.86	Yes	17.55***	No	23.91***	Yes
Aqueous extract 100mg/kg	0.00	Yes	10.17	No	18.53	No	24.40***	Yes
Chitosan based extract 100mg/kg	0.00	Yes	13.10	No	26.03**	Yes	33.78***	Yes
Chitosan based extract 200mg/kg	0.00	Yes	16.55	No	29.04***	Yes	41.05***	Yes

P*** < 0.0001 one way ANOVA followed by tukey post- hoc test.

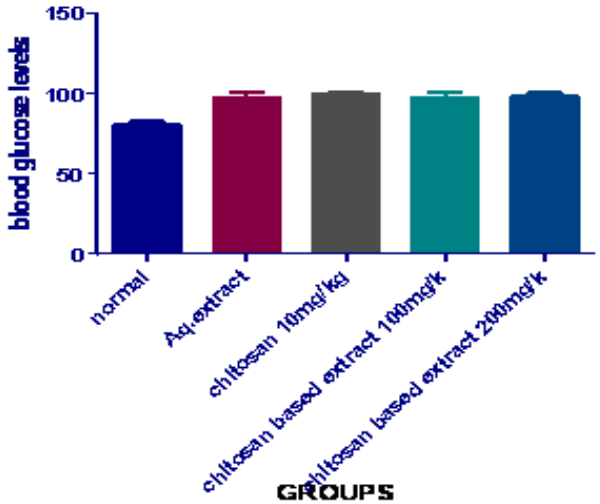

Fig. 2: Comparison of blood glucose levels in different groups at 0 day

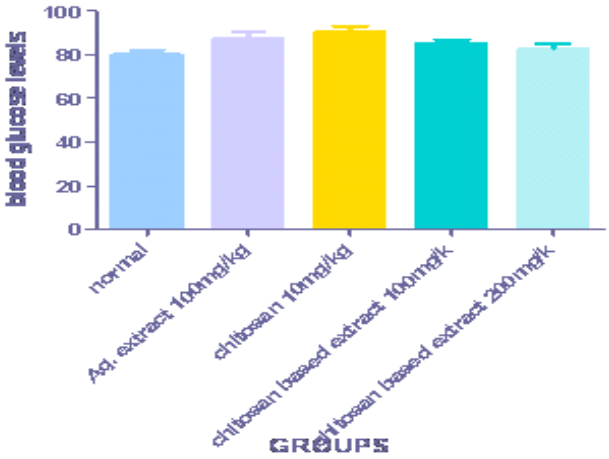

Fig. 3: Comparison of blood glucose levels in different groups at 1st day

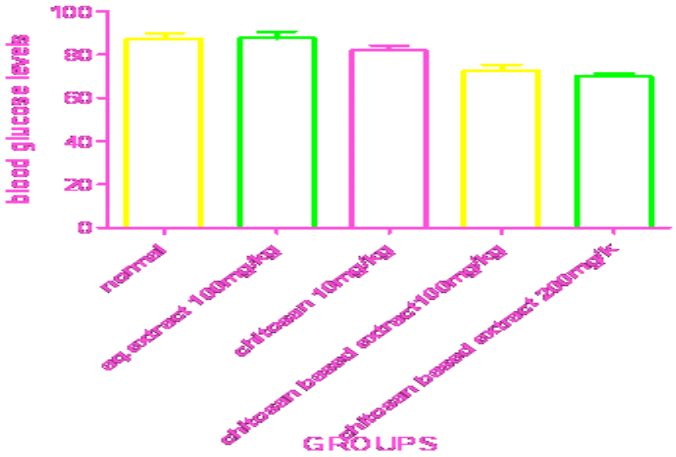

Fig. 4: Comparison of blood glucose levels in different groups at 4th day

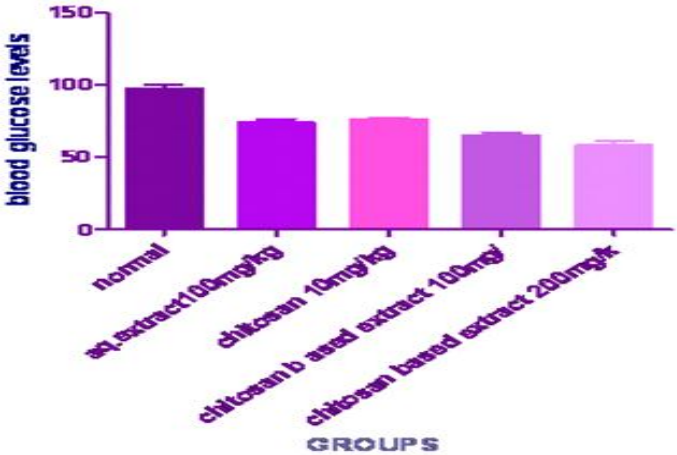

Fig. 5: Comparison of blood glucose levels in different groups at 7th day

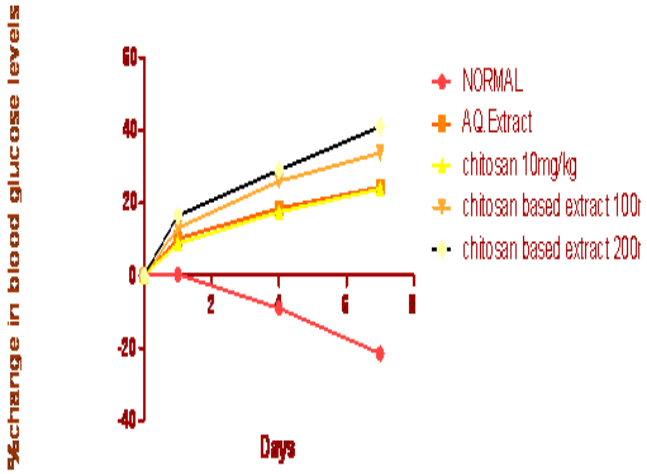

Fig. 6: % Serum glucose reduction produced by chitosan based leaf extract of *Elaeocarpus ganitrus* in normal rats

Table 7: Results of antidiabetic activity in STZ induced rats
The blood glucose levels of normal rats (control, n=6) expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	73	77	90	92
R2	77	79	94	97
R3	79	82	87	91
R4	74	84	88	94
R5	75	81	81	95
R6	84	86	87	97
MEAN ± SEM	77 ± 1.50	81.5 ± 1.21	87.83 ± 1.58	94.33 ± 0.98

Table 8: The blood glucose levels of STZ induced diabetic rats (control, n=6) expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	271	272	292	324
R2	274	281	297	370
R3	267	271	285	327
R4	245	257	274	322
R5	286	287	310	334
R6	279	282	321	341
MEAN ± SEM	270.33 ± 5.23	275 ± 4.00	296.5 ± 6.33	336.33 ± 6.65

Table 9: Effect of aqueous extract of *Elaeocarpus ganitrus* (100mg/kg body weight, oral) on blood glucose in STZ induced diabetic rats expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	274	241	184	132
R2	289	247	187	134
R3	292	269	174	122
R4	285	254	179	124
R5	297	257	187	137
R6	275	245	192	142
MEAN ± SEM	285.33 ± 3.45	252.16 ± 3.77	183.83 ± 2.39	131.83 ± 2.85

In diabetic rats aqueous extract of *Elaeocarpus ganitrus* at the dose level of 100mg/kg body weight produces maximum blood glucose reduction of 11.62%, 35.57% & 51.03 %respectively at 1, 4 & 7 days after administration.

Table 10: Effect of chitosan based extract of *Elaeocarpus ganitrus* (100mg/kg body weight, oral) on blood glucose in STZ induced diabetic rats expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	322	229	157	127
R2	294	227	159	107
R3	287	219	142	112
R4	297	221	144	117
R5	287	217	147	109
R6	312	209	151	108
MEAN ± SEM	299.83 ± 5.29	220.33 ± 2.69	150 ± 2.58	113.33 ± 2.83

In diabetic rats chitosan based leaf extract of *Elaeocarpus ganitrus* at the dose level of 100mg/kg body weight produces maximum blood glucose reduction of 28.07%, 55.42% & 62.20% respectively at 1, 4 & 7 days after administration.

Table 11: Effect of chitosan based extract of *Elaeocarpus ganitrus* (200mg/kg body weight, oral) on blood glucose in STZ induced diabetic rats expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	327	229	132	121
R2	317	227	141	117
R3	297	219	134	118
R4	285	221	139	112
R5	314	217	122	107
R6	298	209	134	114
MEAN ± SEM	306.33 ± 5.79	220.33 ± 2.69	133.66 ± 2.48	114.83 ± 1.84

In diabetic rats chitosan based leaf extract of *Elaeocarpus ganitrus* at the dose level of 200mg/kg body weight produces maximum blood glucose reduction of 34.96%, 55.42 %& 62.51% respectively at 1, 4 & 7 days after administration.

Table 12: Effect of chitosan (10mg/kg body weight, oral) on blood glucose in STZ induced diabetic rats expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	312	289	279	267
R2	295	274	268	259
R3	287	269	257	248
R4	314	284	274	264
R5	297	277	269	257
R6	271	267	259	247
MEAN ± SEM	296 ± 5.97	276.66 ± 3.18	267.66 ± 3.15	257 ± 3.04

In diabetic rats chitosan at the dose level of 10mg/kg body weight produces maximum blood glucose reduction of 6.53%, 9.57% & 13.17 % respectively at 1, 4&7 days after administration.

Table 13: Effect of glimepride (20mg/kg body weight, oral) on blood glucose in STZ induced diabetic rats expressed as mg%

S. No.	0 day	1st day	4th day	7th day
R1	354	212	175	95
R2	243	214	187	94
R3	276	207	169	107
R4	256	209	174	121
R5	279	217	184	98
R6	242	223	175	127
MEAN ± SEM	275 ± 1.55	213.66 ± 2.15	177.33 ± 2.52	107 ± 5.24

In diabetic rats glimepride at the dose level of 20mg/kg body weight produces maximum blood glucose reduction of 22.30%, 35.51%& 61.09% respectively at 1, 4 & 7 days after administration.

Table 15: Comparision of antidiabetic effect of chitosan based leaf extract of *Elaeocarpus ganitrus* with disease control in STZ induced diabetic rats

S. No.	0 day		1st day		4th day		7th day	
	%change in blood glucose	Statistical significance p<0.05	%change in blood glucose	Statistical significance p<0.05	%change in blood glucose	Statistical significance p<0.05	%change in blood glucose	Statistical significance p<0.05
Normal control	0.00	Yes	-5.84***	Yes	-13.63***	Yes	-22.46***	Yes
Tween80	0.00	No	-2.55*	Yes	-3.88	No	-9.39	No
Glimepiride	0.00	No	22.30***	Yes	35.51***	Yes	61.09***	Yes
Chitosan	0.00	No	6.53	No	9.57***	Yes	13.17***	Yes
Aqueous extract 100mg/kg	0.00	N0	11.62**	Yes	35.57***	Yes	53.7***	Yes
Chitosan based extract 100mg/kg	0.00	No	28.07***	Yes	51.03***	Yes	62.20***	Yes
Chitosan based extract 200mg/kg	0.00	Yes	34.96***	Yes	55.42***	Yes	62.51***	Yes

P*** < 0.0001 one way ANOVA followed by tukey post- hoc test.

Table 16: Comparision of antidiabetic effect of chitosan based leaf extract of E*laeocarpus ganitrus* with glimepiride in STZ induced diabetic rats

S. No.	0 day		1st day		4th day		7th day	
	%change in blood glucose	Statistical significance p<0.05	%change in blood glucose	Statistical significance p<0.05	%change in blood glucose	Statistical significance p<0.05	%change in blood glucose	Statistical significance p<0.05
Normal control	0.00	Yes	-5.84***	Yes	-13.63***	Yes	-22.46	No
Tween80	0.00	No	-2.55***	Yes	-3.88***	Yes	-9.39***	Yes
Chitosan	0.00	No	6.53***	Yes	9.57***	Yes	13.17***	Yes
Aqueous extract 100mg/kg	0.00	N0	11.62***	Yes	35.57	No	53.7***	Yes
Chitosan based extract 100mg/kg	0.00	No	28.07*	Yes	51.03***	Yes	62.20	No
Chitosan based extract 200mg/kg	0.00	No	34.96	No	55.42***	Yes	62.51	No

P*** < 0.0001 one way ANOVA followed by tukey post- hoc test.

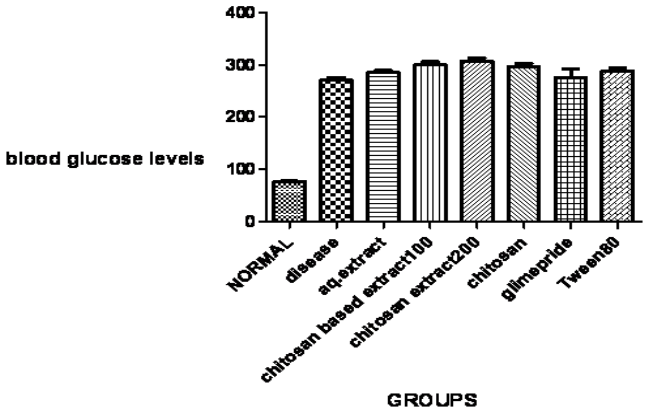

Fig. 7: Comparison of blood glucose levels in different groups at 0 day

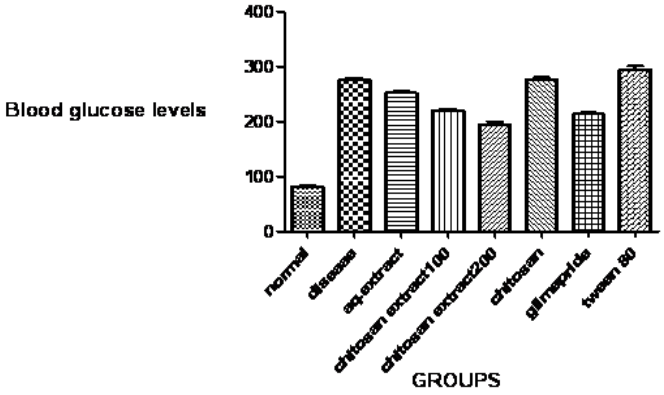

Fig. 8: Comparison of blood glucose levels in different groups at 1st day

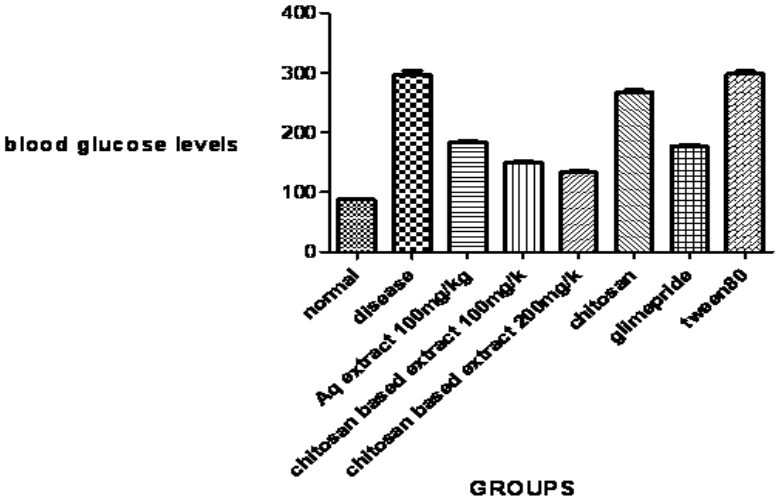

Fig. 9: Comparison of blood glucose levels in different groups at 4th day

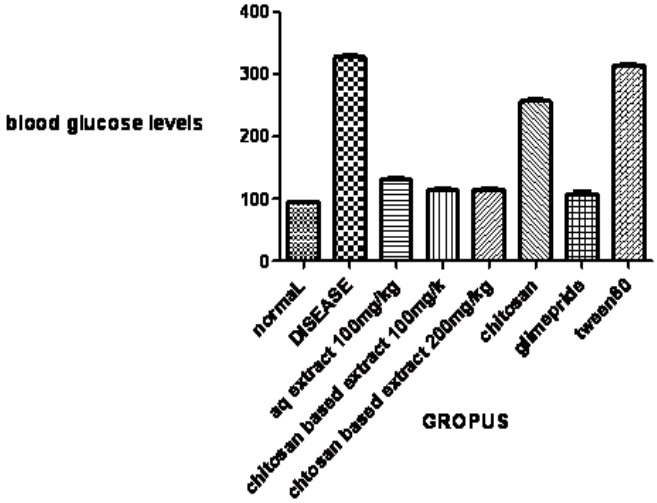

Fig. 10: Comparison of blood glucose levels in different groups at 7th day

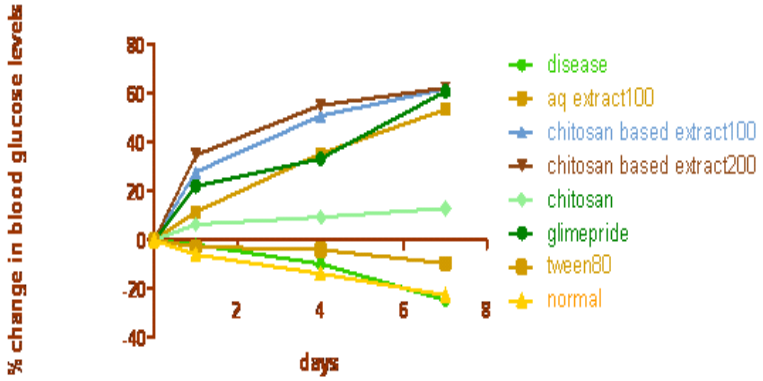

Fig. 11: % Serum glucose reduction produced by chitosan based leaf extract of *Elaeocarpus ganitrus* in STZ induced diabetic rats

DISCUSSION

In the information regarding the use of *Elaeocarpus ganitrus* leaves for several disorders systemic studies were carried out in our laboratory to establish the antidiabetic potential of chitosan based extract of *Elaeocarpus ganitrus* and antidiabetic activity of aqueous extract of *Elaeocarpus ganitrus*. In the present study rats were use as experimental animals.

It was found that chitosan based leaf extract of *Elaeocarpus ganitrus* produced hypoglycaemic effect in normal rats. Chitosan based aqueous extract at doses of 100 mg/kg body weight orally produced clinically significant hypoglycaemia of 13.10%, 26.03% and 33.78% respectively at 1st, 4th & 7th days of administration in normal rats. Chitosan based aqueous extract of 200 mg/kg body weight orally produced clinically significant hypoglycaemia of 16.55 %, 29.04 % and 41.05% respectively at 1st, 4th & 7th days of administration in normal rats. The aqueous extract of *Elaeocarpus ganitrus* at a dose of 100 mg/kg produced clinically significant hypoglycaemia of 10.17%, 18.53% & 24.40% respectively at 1st, 4th and 7th days of administration in normal rats. Chitosan at a dose of 10 mg/kg also produced clinically significant hypoglycaemia of 8.86%, 17.55% &

23.91% respectively at 1st, 4th and 7th days of administration in normal rats. The onset of action was approximately at 1st day. Duration and intensity of action increased with the addition of doses.

It was found that chitosan based extract of *Elaeocarpus ganitrus* produces antidiabetic effect in diabetic rats in different doses. Chitosan based extract of *Elaeocarpus ganitrus* at doses of 100, 200 mg/kg body weight orally produced clinically significant antidiabetic activity respectively and their peak activities were registered on 7th day after administration. The onset of action was approximately at 1st day. Duration and intensity of action increased with the addition of doses.

Elaeocarpus ganitrus leaf extract produces antidiabetic effect in streptozotocin induced diabetic rats. *Elaeocarpus ganitrus* at dose of 100mg/kg body weight produced blood glucose reduction of 11.62%, 35.57% & 53.7 %respectively at 1, 4 & 7 days after administration. And their peak activities were registered at 7th day after oral administration. The onset of action was approximately at 1st day. The doses which produce approximately 30% blood glucose reduction are usually sufficient to control blood sugar level in moderate diabetics.

The effect of aqueous extract of *Elaeocarpus ganitrus* on blood glucose levels in diabetic rats was found to be higher to that of normal rats. However slight variation (quantitative) in percent reduction was noticed. Since the selected extract posses antioxidant activity they can antagonize the hyperglycaemia induced stress and improve the condition[9]. Since the chemical constituents in the plant extract were identified to be responsible for antidiabetic activity in earlier studies, such compounds along with others might be responsible for the antidiabetic activity.

The effect of chitosan based extract of *Elaeocarpus ganitrus* on blood glucose levels in diabetic rats was found to higher to that of normal rats. However variation in percentage reduction was noticed. Since the selected extract posses antioxidant activity and Chitosan could increase insulin secretion of pancreatic cells and improve the overgrowth of cells and isolated pancreatic islet cells, decrease and normalize the disorders of glucose tolerance[10].

Since the chitosan based extract produced clinically significant antidiabetic activity, it was standardised by comparing with known standard drug, glimepiride in diabetic rats. Glimepiride is a medium-to-long acting sulfonylurea antidiabetic drug. Glimepiride is the first third-generation sulfonylurea. Glimepiride lowers the blood glucose level by stimulating pancreatic beta cells to produce more insulin and by inducing increased activity of intracellular insulin receptors. A dose of 100 mg/kg body weight of chitosan based extract of *Elaeocarpus ganitrus* produces comparable effect with 20 mg/kg body weight of glimepiride on oral administration which produced maximum serum glucose reduction of 62.20% in streptozotocin induced diabetic rats. A dose of 200 mg/kg body weight of chitosan based extract of *Elaeocarpus ganitrus* produces comparable effect with 20 mg/kg body weight of glimepiride on oral administration which produced maximum serum glucose reduction of 62.51% in streptozotocin induced diabetic rats.

CONCLUSION

The study indicates clinically significant antidiabetic activity of *Elaeocarpus ganitrus* in diabetic rats. The chitosan based extract improved the antidiabetic activity of *Elaeocarpus ganitrus* clearly indicating synergism. The % blood glucose reduction of the chitosan based aqueous extract at a dose of 200mg/kg is comparable with that of standard anti diabetic drug glimeperide 20 mg/kg. This reduction of blood glucose is more in diabetic rats when compared to normal rats treated with the same dose.

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