Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 26-31
Received: 10-03-2020
Accepted: 14-04-2020

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Evaluation of comparative Hypoglycaemic activity of ethanolic leaves extracts of datura stramonium and Eclipta alba

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DOI: https://doi.org/10.22271/phyto.2020.v9.i3a.11475

Abstract
Datura stramonium is a yearly plant belongs to family Solanaceae. *Datura stramonium* is generally known as jimson weed or thorn apple. Eclipta alba is commonly known as False Daisy or Bhringraj, a plant having a place with the family Asteraceae. Diabetes was produce in overnight fasted male Wistar rats (160-250 mg/kg) by Interparitonial injection of 150 mg/kg body weight of alloxan. Alloxan monohydrate 150 mg/kg, b.w was dissolved in normal saline and injected I.P after 18 hours fasting. The rats were then given 4% w/v glucose solution in feeding bottle for the next 24 h in their cages to inhibit hypoglycemia. The rats will be divided into 7 groups of 6 animals each, total 42 animals used. Ethanolic extract of both plants (datura stramonium and eclipta alba) given in dose of 100, 200 mg/kg b.w and 200, 400 mg/kg b.w ethanolic extracts of both plants are administered orally for 14 days, in a single dose daily seven days after confirmation of hyperglycemia. Standard drug Glibenclamid & ethanolic leaves extracts of datura stramonium and eclipta Alba were administered orally for 14 days, in a single dose daily 7 days after confirmation of hyperglycemia. Significantly reversed decrease in body weight seen in diabetes. The dose of 200, mg/kg b.w /day of ethanolic extract of leaves datura and 400 mg/kg b.w/day of ethanolic extract of eclipta alba leaves were found to be having maximum activity.

Keywords: Diabetes mellitus, eclipta alba, datura stramonium, hypoglycemic effect, rats

Introduction
Ayurvedic approach towards diabetes and every other illness is that they are brought about by doshic unbalance. In 1674 a doctor named Willis instituted the expression "Diabetes Mellitus" (from the Greek word for nectar). Diabetes Mellitus (DM) is a gathering of metabolic issue described by hyperglycemia and anomalies in starch, fat and protein digestion. The quantity of individuals with diabetes in expanding because of populace development, maturing, urbanization, and expanding pervasiveness of heftiness and physical dormancy, the significant point of diabetes the executives are to forestall optional complexities. In old time, type 2 diabetes was known as an infection of raised glucose levels related with weight. Diabetes Mellitus (DM) is a turmoil that influences the body's capacity to make or use insulin. Insulin is a hormone created in the pancreas that helps transport (glucose) from the circulatory system in to the cells so they can separate it and use it for fuel. As indicated by the Internationals Diabetes Federation (IDF), the general cost appraisal for the worldwide avoidance and treatment of diabetes will approach US$490 billion by 2030. India has the most noteworthy number of diabetic patients, and India is being known as the diabetic capital of the world. Studies have demonstrated a noteworthy age - related pervasiveness in the urban populaces, to a great extent among the individuals with inactive way of life.
Datura stramonium is a yearly plant belongs to family Solanaceae. The occurrence of *D. stramonium* harming is sporadic with a group of harming cases happening for the most part among youths. *Datura stramonium* is generally known as jimson weed or thorn apple. *Eclipta alba* is commonly known as False Daisy or Bhringraj, a plant having a place with the family Asteraceae. It is usually found on poorly drained, wet areas; along streams and ditches in marshes and on the dikes of rice paddies.

Materials and Methods
Materials: The whole plant of *datura stramonium* and *eclipta alba* was collected during the month of May to June from the Gwalior, and Morena district of M.P. Identified and authentificated by Prof. P Jayaraman, Ph.D., director (Retd, professor, Presidency College Chennai-5) plant anatomy research center Chennai (reg.no. PARC/2019/4190)

Preparation of extract
The crude leaves of the plant assembled were sun dried and processed into fine powder by many cycles of grinding and sieving. 250 g of the fine powder was extracted with 60% ethanol using Soxhlet extractor. The percentage recovery was calculated to be 13% w/w. Used for hypoglycemic activity studies extract obtain was store under refrigerating position.

Experimental Animals
The complete course of experiment was carried out using healthy male Wistar rats weighing between 150-200 gm were obtained from animal house, ShriRam College of Pharmacy, Banmore, M.P, India. They were housed in standard laboratory condition at room temperature along with 12hour light/dark rounds. Animals were provide with standard pelleted diet obtained commercially from the manufacture and water ad libitum. Later 7 days of acclimatization period, they were randomly chosen for different trial groups. Ethical clearance was obtained from Institutional Animal Ethics Committee (891/PO/Re/S/OS/CPCSEA). ShriRam College of pharmacy, Banmore, before conducting the experiment

Induction of diabetes
Diabetes was produce in overnight fasted male Wistar rats (160-250 mg/kg) by interparitonial injection of 150 mg/kg body weight of alloxan. Alloxan monohydrate 150 mg/kg, b.w was dissolved in normal saline and injected I.P after 18 hours fasting. The rats were then given 4% w/v glucose solution in feeding bottle for the next 24 h in their cages to inhibit hypoglycemia. After 48 hrs, alloxinization in blood samples collected by tail tipping method using glucometer, rats with marked hyperglycemic fasting blood glucose more than 200 mg/dl were selected and used for the study. All the animals were allowed free access to water, pellet diet and keep at room temperature in poly-ethylene cages

Experimental study design:
The rats will be divided in to 7 groups of 6 animals each.

- **Group I:** Normal control administered with 0.9% sodium chloride (NACL**)**
- **Group II:** Alloxan induced diabetic control administered with 0.9% NACL
- **Group III:** Alloxan induced diabetic control administered with Glibenclamid (GLB) at 10 mg/kg b.w.
- **Group IV:** Ethanol leaf extract of datura stramonium (100 mg/kg)
- **Group V:** Ethanol leaf extract of datura stramonium (200 mg/kg)
- **Group VI:** Ethanol leaf extract of eclipta alba (200mg/kg)
- **Group VII:** Ethanol leaf extract of eclipta alba (400mg/kg)

Selection of animal species
Healthy male Wistar rats at the start of its dosing, each animal should be between 8 and 12 weeks old and its weight should fall in an interval between 150-200 gm.

Method
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Housing and Feeding Condition
The temperature in the experimental animal room should be 25 ºC. Supposing the relative humidity should be at least 30% and select not exceed 70% other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the continuity being 12 hours light and 12 hours dark. For feeding, conventional rodent laboratory diet may used with an ultimate supply of drinking water. The animals are randomly choice, symbol to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Administration of doses
The test substances are administered in a single by gavages using stomach tube. The animals were fasted prior to dosing, following period fasting, the animal were weighed test substances was administered.

Number of animals and dose levels
The rats will be divided into 7 groups of 6 animals each, total 42 animals used. Ethanolic extract of both plants (*Datura stramonium* and *Eclipta alba*) given in dose of 100, 200 mg/kg b.w and 200, 400 mg/kg b.w ethanolic extracts of both plants are administered orally for 14 days, in a single dose daily seven days after confirmation of hyperglycemia.

Parameters
Blood glucose
The treatment was started from the same day except normal control and diabetic control groups for period of 14 days orally. During this period, animal, in all groups had free access to standard diet and water. Blood glucose levels were estimated on 1st, 5th, 10th and 14th day of the treatment. Blood samples were collected from the tail vein and glucose levels were estimated using glucometer strip and a glucometer (Jyoti scientific laboratories).

Body weight
Decrease in body weight seen in diabetes. The body weights of the rats were recorded on 1st, 5th, 10th and 14 day of the treatment with the help of electronic balance.

Biochemical parameters
For estimation of biochemical parameters on day 15th blood was collected from retro orbital plexus and centrifuge for serum separation.

Total cholesterol
Total cholesterol was measured by using ERBA diagnostic Kits.

Clinical significance: Measurement of serum cholesterol levels is useful in evaluation of the risk of the coronary arterial occlusion, atherosclerosis, myocardial infarction and liver function.

Increase: Levels increase in primary hyper lipoproteinaemias, nephritic syndrome, obstructive jaundice and diabetes mellitus.

Decrease: Level decrease in anemia's, hemolytic jaundice, severe malfunction, acute infections and in terminal state.

Estimation of Total Protein (TP)

Clinical significance

Total protein is useful for monitoring gross change in protein levels caused by various disease states. It is usually performed in conjugation with other tests such as serum albumin, protein electrophoresis.

Increased: ↑ed levels are found mainly in dehydration

Decreased: ↓ed levels are found mainly in malnutrition, impaired synthesis, proteins losses as in hemorrhage or excessive protein catabolism

Methodology

The peptide bonds of protein react with copper II ions alkaline solution to form blue violet complex, (biurate reaction). Each copper ion complexing with 5 or 6 Peptide bonds, tartarate added as a stabilizer whilst iodide is used to prevent auto oxidation of the alkaline copper complex. The color is proportional to the protein concentration and is measured at 546 nm (520-560).

Result and Observations

Table 1: Effect of ethanolic leaves extract *of datura stramonium* and *eclipta alba* in diffrent animal groups

group	Treatment	Fasting blood glucose level (mg/dl)			
		day 1	day 5	day 10	day 14
group I	normal control	85.08±4.36	86.17±4.42	84.81±5.95(NS)	84.71±6.12(NS)
group II	diabetic control	261.48±8.36	264.27±8.28	268.02±8.47(NS)	271.33±8.16(NS)
group III	alloxan + Glibenclamid	200.36±5.24	141.19±2.43	124.51±2.00*	114.83±3.21*
group IV	alloxan + ethanolic datura leaves extract (100 mg/ kg b.w)	204.39±4.03	180.85±2.31	144.55±2.54*	142.82±2.75*
group V	alloxan + ethanolic datura extract leaves (200 mg/ kg b.w)	207.25±4.26	146.97±2.246	125.22±2.84*	114.52±2.54*
group VI	alloxan + ethanolic eclipta alba leaves extract (200 mg/kg b.w)	212.15±4.87	180.43±2.46	163.45±2.73*	156.62±1.61*
group VII	alloxan + ethanolic eclipta alba leaves extract (400 mg/kg b.w)	208.25±4.26	178.64±1.54	132.36±3.41*	114.83±.52*

Alloxan monohydrate (150 mg/kg) was administered i.p, in sterile saline, single dose 7 days before the administration of different ethanolic extracts.

Standard drug Glibenclamid & ethanolic leaves extracts of datura stramonium and eclipta alba were administered orally for 14 days, in a single dose daily seven day after confirmation of Hyperglycemia.

N=6 (no of animals in each group)

Statically significance test was done by one way ANOVA followed by Dunnett's test using SPSS16.0 window version.

*p< 0.05 compared to disease control group.

Table 2: Effect of ethanolic leaves extract of *datura stramonium* and *eclipta alba* in diffrent animal groups

Group	Treatment	Body weight of animals (g)			
		day 1	day 5	day 10	day 14
group I	normal control	228.86±2.76	229.84±2.77	233.01±2.81	222.04±2.85
group II	diabetic control	180.57±8.36	163.88±2.58	152.22±2.52*	124.77±1.73*
group III	alloxan + Gilbenclamide	183.82±2.85	173.86±2.65	152.96±2.03	137.51±1.91*
group IV	alloxan + ethanolic datura leaves extract (100 mg/ kg b.w)	183.87 ±2.78	174.71±2.42	165.51±2.34*	162.37±1.73*
group V	alloxan + ethanolic datura extract leaves (200 mg/ kg b.w)	188.57±2.38	185.47±2.16	179.56±1.88*	172.63±1.25*
group VI	alloxan + ethanolic eclipta alba leaves extract (200 mg /kg b.w)	193.89±2.46	184.53±2.06*	176.45±1.68*	176.42±1.51*
group VII	alloxan + ethanolic eclipta alba leaves extract (400 mg/kg b.w)	156.38±1.46	153.96±1.74	148.16±2.52	150.61±.58

Alloxan monohydrate (150 mg/kg) was administered i.p, in sterile saline, single dose 7 days before the administration of different ethanolic extracts.

Standard drug Glibenclamid &ethanolic leaves extracts of *datura stramonium* and *eclipta alba* were administered orally for 14 days, in a signal dose daily seven day after confirmation of hyperglycemia.

N=6 (no of animals in each group)

Statically significance test was done by one way ANOVA followed by Dunnett's test.

*p< 0.05 compared to disease control group.

Table 3: Effect of ethanolic leaves extract of *datura stramonium* and *eclipta alba* in diffrent animal groups.

Group	Treatment	Serum cholesterol (mg/dl)	Total serum protein (g/dl)
group I	normal control	106.41±2.07	6.17±0.17
group II	diabetic control	179.81±1.45	4.71±0.95
group III	alloxan + Glibenclamid	121.91±*	6.11±0.17*
group IV	alloxan + ethanolic datura leaves extract (100mg/ kg b.w)	170.86±2.78	4.96±0.12
group V	alloxan + ethanolic datura extract leaves (200mg/ kg b.w)	145.58±2.39*	5.61±0.16*
group VI	alloxan + ethanolic eclipta alba leaves extract (200 mg/kg b.w)	168.54±2.88	5.14±0.55
group VII	alloxan + ethanolic eclipta alba leaves extract (400mg/kg b.w)	145.57±2.58*	5.63±0.32*

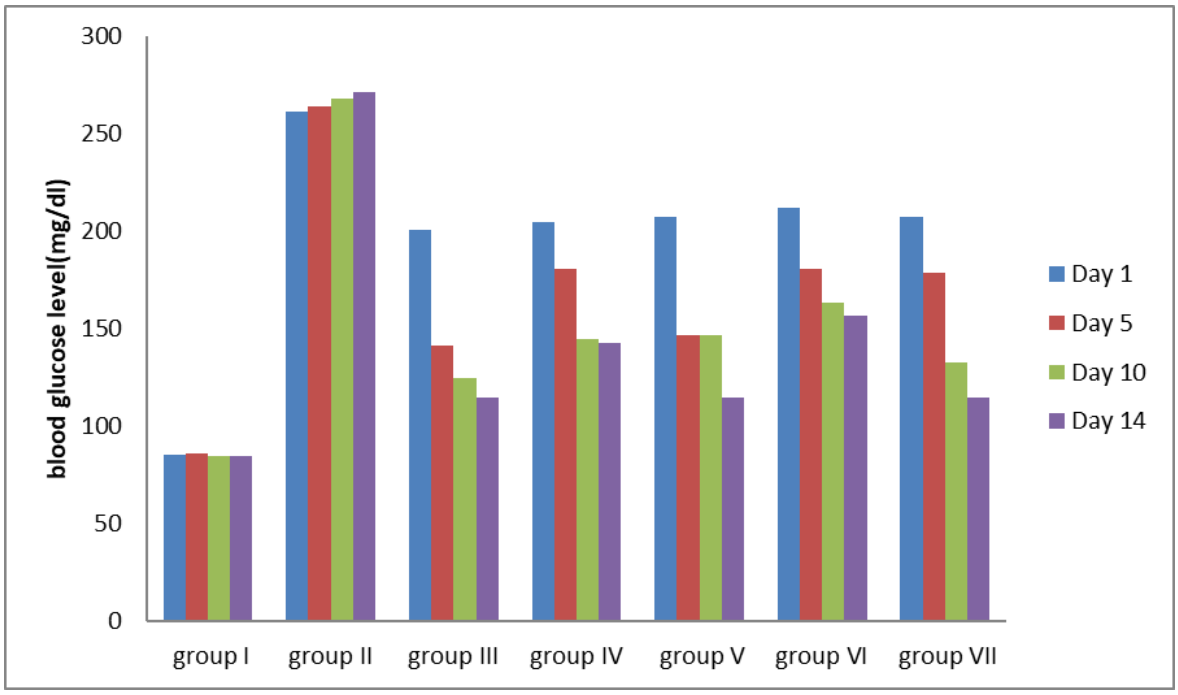
Alloxan monohydrate (150 mg/kg) was administered i.p, in sterile saline, single dose 7 days before the administration of different ethanolic extracts.

Standard drug Glibenclamid & ethanolic leaves extracts of *datura stramonium* and *eclipta alba* were administered orally for 14 days, in a single dose daily 7 days after confirmation of hyperglycemia.

N=6 (no of animals in each group)

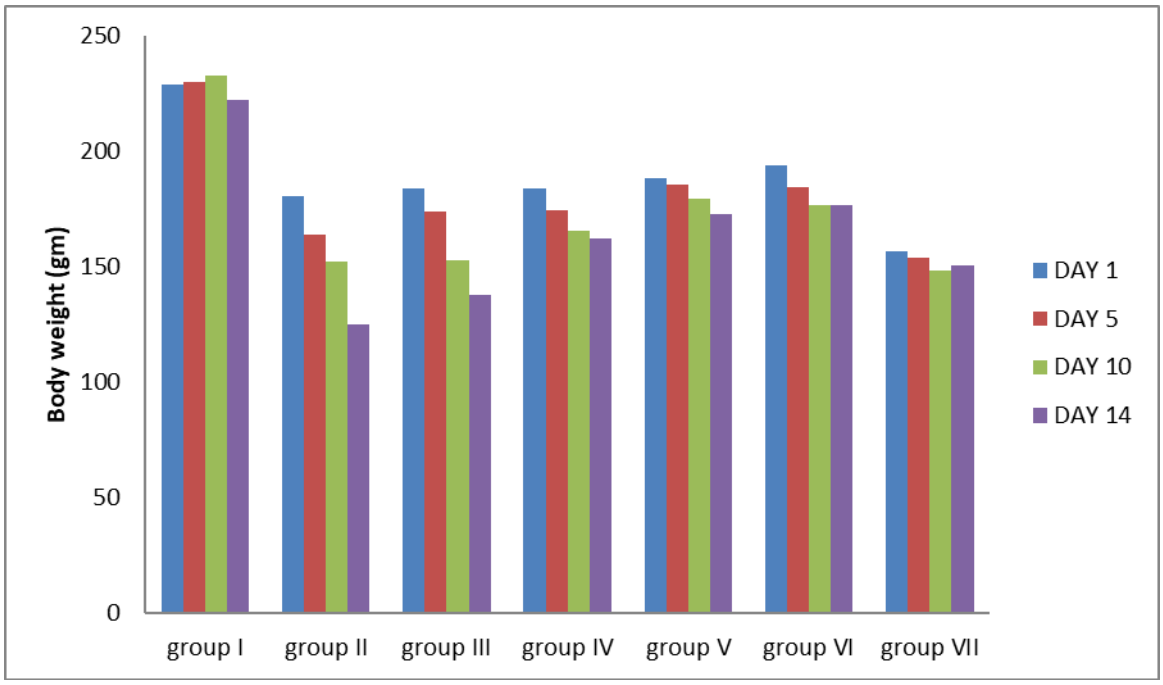
Statically significance test was done by one way ANOVA followed by Dunnett's test using SPSS16.0 window version.

*p< 0.05 compared to disease control group.



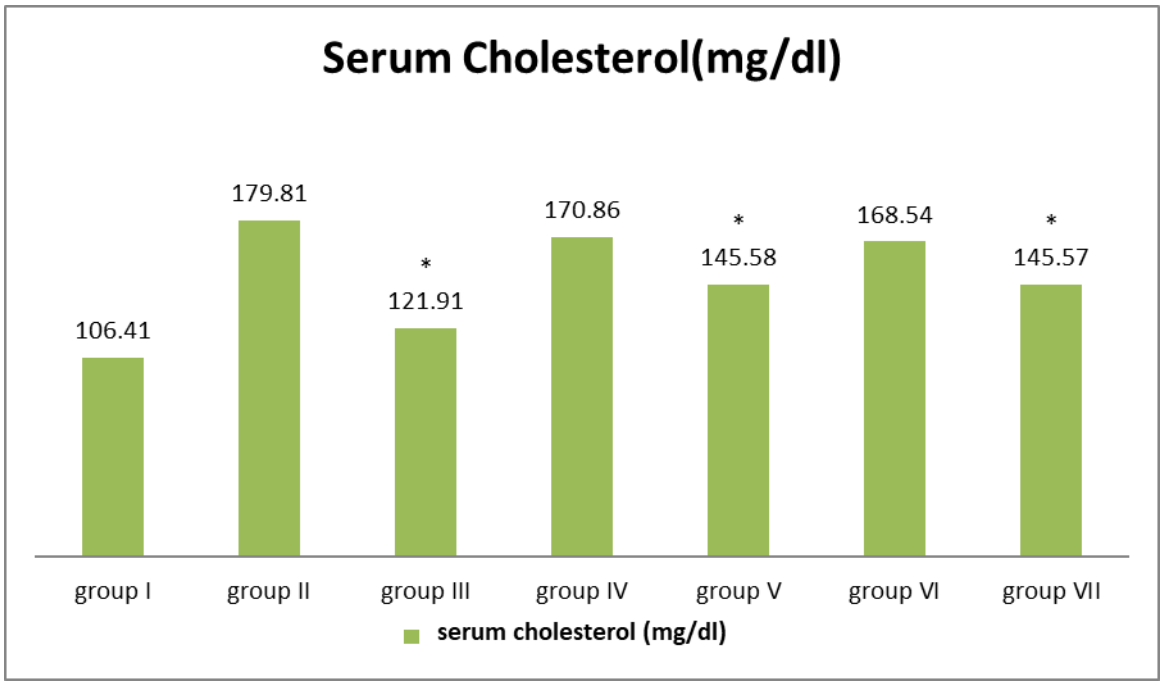
Statically significance test was done by one way ANOVA followed by Dunnett's test.
*p<0.05 compared to disease control group.
Control value for Body weight -228.86±2.76
All values are MEAN± SEM of 6 animals per group

Fig 1: Effect of ethanolic leaves extract of *datura stramonium* and *eclipta alba* in different animal groups. Fating blood glubcose level (mg/dl)



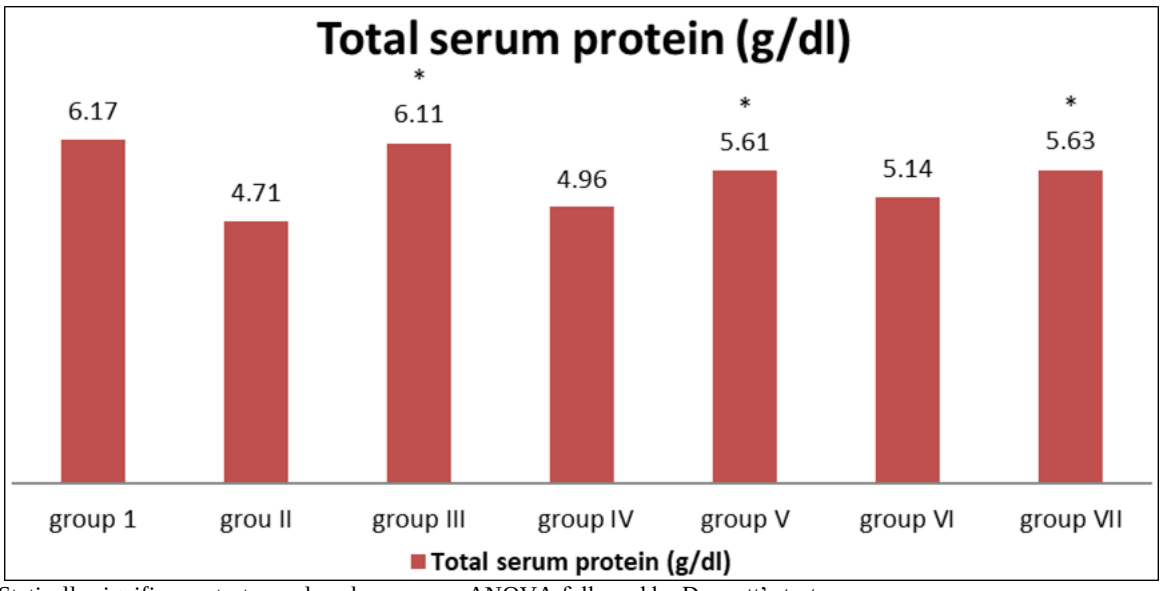
Statically significance test was done by one way ANOVA followed by Dunnett's test
*p< 0.05 compared to disease control group.
Control value for blood glucose level- 85.08±4.34
All values are MEAN± SEM of 6 animals per group

Fig 2: Effect of ethanolic leaves extract of datura stramonium and eclipta alba in different animal groups. Body weight of animal (gm)



Statically significance test was done by one way ANOVA followed by Dunnett's test.
*p< 0.05 compared to disease control group.
Control value for serum cholesterol-106.41±2.07 (mg/dl)
All values are MEAN ± SEM 6 Animal per group

Fig 3: Effect of ethanolic leaves extract of *datura stramonium* and *eclipta alba* in different animal groups.



Statically significance test was done by one way ANOVA followed by Dunnett's test.
*p< 0.05 compared to disease control group.
Control value for serum cholesterol- 6.11±0.17*(mg/dl)
All values are MEAN ± SEM 6 Animal per group

Fig 4: Effect of ethanolic leaves extract of *datura stramonium* and *eclipta alba* in different animal groups.

Conclusion

Alloxan (150 mg/kg b. w) were found to induce in rat s evidenced by the increased blood glucose levels. Alloxan induced diabetes mellitus (14 days study). Ethanolic leaves extract of *datura stramonium* and *eclipta alba* Revealed a dose dependant antidiabetic potential in rats with doses of 100, 200 and 200, 400 mg / kg b.w the dose of 400 mg\kg b.w / day of ethanolic extract of leaf was found to be having maximum activity, and the effect was seen equal to the levels of blood glucose with std. Antidiabetic drug, Glibenclamid. And the value was nearly equal to normal control levels. The dose of 200 mg/kg b. W / day of ethanolic extract of *datura* leaves and 400 mg/kg b.w /day of ethanolic concentrated of *eclipta* leaves were also found to be having hypoglycemic activity.

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