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HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECT OF METHONOLIC SEED EXTRACT OF *EMBELIA ROBUSTA* IN ALLOXAN INDUCED DYSLIPIDEMIA IN WISTAR RATS

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ABSTRACT
Ayurveda refers *Embelia robusta* (Sanskrit: *Tandula*) as a medicinal plant used for the treatment of diabetes mellitus, anticancer, antiinflammatory, antifungal, antibacterial. **Objective:** The present study was carried out to evaluate the hypolipidemic activity of methonolic seed extract of *E.robusta* in normal and Alloxan induced diabetic dyslipidemic rats. **Method:** Diabetes was induced in male wistar rats by single intraperitonial injection of Alloxan (130mg/kg) body weight. After diabetic induction methanolic seed extract of *E.robusta* at the dose 50mg/kg (T1) and 100mg/kg (T2) body weight was used as a test drug and Gliclazide 50mg/kg body weight as a standard drug to the diabetes induced experimental rats for 30days. Acute toxicity and Oral glucose tolerance study was carried out in normal rats, Activity of methonolic seed extract of *E.robusta* on lipid profile was carried on diabetes induced rats, which showed significant reduction in lipid parameters in both Test group T1 & Test group T2. **Result**: Acute toxicity study showed no signs of toxicity by methanolic seed extract of *E.robusta* even when the dose was increased to 4000mg/kg body weight. In oral glucose tolerance test rats treated with methanolic seed extract of *E.robusta* at the dose T2 showed significant ($P<0.05$) reduction in hyperglycaemic control in Group-IV and there was significant reduction in serum level TC (116.3), TG (108.7), LDL (36.43), VLDL (21.7) and increases in level of HDL (58.17), comparable to the standard control. **Conclusion:** The results indicate that methonolic seed extract of *E.robusta* has hypoglycemic and hypolipidemic activity.

KEYWORDS: *E. robusta*, Alloxan, Acute toxicity, Oral glucose tolerance, Hypolipidemia.

INTRODUCTION
Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia resulting from defective insulin secretion due to β-cell dysfuction, resistance to insulin action. Increase in adipose tissue mass leads to obesity associated with hyperlipidemia. Hyperlipidemia has been ranked as one of the greatest risk factors associated with lipid disorder and is considered to cause the atherosclerotic cardiovascular disease and hyperlipidemic stroke.[1] Hyperlipidemia is characterized by elevated serum total cholesterol and low density and very low density lipoprotein cholesterol and decrease high density lipoprotein.[2] Treatment of hyperlipidemia in diabetes involves improving glycemic control, regular exercise and using lipid-lowering diet and drugs.[3] Currently available hypolipidemic drugs have been associated with number of side effect. The long term consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing dry skin and abnormal liver function.[4]

Indian traditional system of Ayurveda is the richest and oldest medicine around the world. There is enough textual evidence of traditional use of medicinal plants as there are more than 1200 anti diabetic plants described in the scientific literature.[5] Medicinal plants contain a large number of active compound like glycosides, alkaloids, terpenoids, flavonoids etc, Which have the pharmacological action and also antilipidemic properties.[6]

Even herbal medicines are used successfully as medicine,[7,8] which are less toxic, free from side effect, cheap and locally available.

E.robusta commonly known as Vidanga or Vayu vidanga belongs to the family Myrsinaceae. It is widely distributed in India, Sri Lanka, Malaysia and South China. It is a woody shrub with long slender brittle stem, long internodes. The leaves are elliptic, broad and covered with minute glands. The flowers are small with white racemes arranged in panicle inflorescence at the

end of the branches. The fruits are berries, round, red to black in color and 2-4mm in diameter. The fruits look like pepper and are often called to as false pepper. The fruits are used as an antihelmintic, diuretic, carminative, contraceptive, anti-bacterial, anti-inflammatory,anti astringent and also fruit decoction is useful in fever and diseases of chest and skin. Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) which is a compound isolated from *E.robusta* Roxb is used in the treatment of antifertility, anti-inflammatory, antioxidant and antitumor properties.[9] So the present study was undertaken to make use of naturally occurring seed of *E.robusta* ROXB and evaluating methanolic seed extract for its hypoglycemic and -hypolipidemic properties in alloxan induced diabetic rats.

MATERIAL AND METHODS

Chemicals

All reagents used were of analytical grade. Alloxan Monohydrate was purchased from Sigma Aldrich, USA.

Plant Material

E.robusta seeds were identified and purchased from local market by Department of Botany, Osmania University and University College for Women, Koti, Hyderabad.

Preparation of Plant Extract

E.robusta seeds were collected, shade dried, finely powdered, seived and extracted twice with methanol for 72hr at 60°c. The extract was evaporated under rotary evaporator and resuspended in distilled water before its use for animal experimentation.

Animal Model

The animal models used for the in vivo study (Male Wistar Rats, weighing 200-250 g) were purchased from National Institute of Nutrition, Hyderabad, Telangana State, India. The animals were grouped and housed in polypropylene cages in an animal room, maintained under laboratory conditions (Temperature 25±2°c) with 12hr day-12hr night cycle. The animals were provides with rodent pellet diet and water adlibitum.The animal study was approved by Institutional Animal Ethics Committee (IAEC) (Registration No.428/01C/CPCSEA, 7$^{t\ h}$ February 2015) Gandhi Medical College, Secunderabad, Hyderabad.

Body Weight Study

Total body weight of diabetic and non diabetic rats were determined using digital balance before and after the experimental period, Initial and final body weight was recorded respectively.

Toxicity Study

After 7 days of acclimatization period, toxicity study of methanolic seed extract of *E.robusta* was carried out in rats. The animals were kept fasting for 12hr and provided only water. The weight of animals were recorded, divided into 3-Groups and treated orally with methanolic seed extract of *E. robusta* at the dose of 50 and 100 mg/kg body weight. Animals were continuously monitored for first hour and every fourth hourly and finally for 24 hrs. Subsequently observations were done for any behavioural changes like grooming, hyperactivity, convulsion and lethality developing during the next 48 hrs.

Oral glucose Tolerance Test

The experimental animals were divided in to 4-groups with 6 animals in each group.

Group -I: Control rats received 1% Tween 80 in distilled water.

Group -II: Standard control (Gliclazide 50mg/kg).

Group -III: Test Group-I (T1) treated with Methanolic seed extract of *E. robusta* at the dose of 50mg/kg body weight.

Group -IV: Test Group-II (T2) treated with Methanolic seed extract of *E. robusta* at the dose of 100mg/kg body weight.

Approximately 2g/kg body wt of glucose solution was loaded orally to all the rats using intragastric tube. After the toxicity study period, on 7th day the rats were fasted overnight with free access to water, fasting blood sample was collected at time intervals of 60,120,180th minute from the tail and blood glucose was measured by using glucometer. Integrated area under curve for glucose (AUC glucose) was calculated by trapezoid rule.[10]

Drug Administration

After inducing diabetes by Alloxan (130 mg/kg body weight), treatment was started with methonolic seed extract of *E.robusta* at the dose of 50mg/kg and 100mg/kg body weight orally using intragastric tube.

Experimental induction of Diabetes

Single dose of 130mg/kg body weight Alloxan monohydrate freshly dissolved in citrate buffer (PH 4.5) was injected intraperitonially into 18 hrs fasted rats. Then animals were given 5% glucose solution to overcome drug-induced hypoglycaemia. After 48 hrs the fasting blood glucose levels were determined by GOD-POD method (11). The rats showing fasting blood glucose more than 200 mg/100 dL were considered as diabetic and selected for the experimentation. Blood lipid levels (TC, TG, HDL, VLDL, LDL) were measured and found to be higher than before induction.

Experimental Design

In the experiment all animals were randomly divided in to 5-Groups with 6 animals in each group.

a. **Group- I** : Normal healthy controls.
b. **Group- II:** Diabetic Control.
d. **Group- III :** Standard; (Gliclazide 50mg/kg body wt) drug was orally fed daily using an intragastric tube for 28 days.
e. **Group -IV:** Test Group- I (T1), treated with methanolic seed extract of *E. robusta* at the dose 50mg/kg body weight was fed to rats daily using an intragastric tube for 30 days.

f. Group - V: Test Group- II (T2), treated with methanolic seed extract of *E. robusta* at the dose 100mg/kg body weight was fed to rats daily using an intragastric tube for 30 days.

Blood Sample collection

Fasting blood samples were collected from retro-orbital plexus using micro-capillary technique under mild ether anesthesia and subjected to estimation of fasting blood glucose on 0^{th}, 7^{th},14^{th},21^{st} ,30^{th} day of the experiment by GOP-POD enzymatic method (10), Serum lipid profile levels were estimated on day 1 and day 30.

Biochemical Analysis

Serum was separated from whole blood sample by centrifugation at 2000 rpm for 20 minutes and used for analysis of TC, TG, HDL, LDL, and VLDL.

Statistical Analysis

All the data were expressed as mean ± standard deviation. The results obtained from the present study were analyzed using One-way ANOVA followed by column statistic comparison tests. Differences between the groups were considered significant at P<0.05.

RESULTS

Body Weight

Administration of methonolic seed extract of *E. robusta* at the dose 50mg/kg and 100mg/kg body weight in Group IV and Group –V showed increased body weight of 3.15 and 5.44% respectively and standard control (7.13%) in (Group-III) showed significant increase (P<0.01) in the body weight in comparison to diabetic control showing significant decrease (P<0.01) in the body weight. (Table:1, Fig:1).

Toxicity studies Result

The toxicity studies showed that the rats have no significant behavioural changes up to 4hrs and no mortality up to 24hrs and then monitored daily for the next 28 days using methonolic seed extract of *E.robusta* extract at the dose of 50mg/kg & 100mg/kg body weight This experiment indicate that *E.robusta* is safer as no lethality and no toxic reactions were observed up to the end of the study period even at the dose of 4000mg/kg body weight showed in (Table:2, Fig:2).

Oral glucose Tolerance test

Oral administration of glucose (2 g/kg body weight) produced significant changes in blood glucose level in normal rats. The Peak increase in mean plasma glucose at 60min in normal control, it was 176.0 mg/dl. Gliclazide treated animals has shown only 96.00mg/dl, seed extract at the dose 50mg/kg is 168.2mg/dl and 100mg/kg is 162.8mg/dl, significantly shown plasma glucose at peak.

In addition estimation of integrated AUC glucose Standard control has shown 36.53% (925.3 ± 11.95), seed extract at the dose 50mg/kg has shown 41.143% (1306 ± 14.89), at the dose 100mg/kg has shown 31.41% (1216 ± 18.30), the total AUC compared with standard control (Table:3, Fig:3).

Hypoglycemic Activity in wistar rats

Table 1: shows the fasting blood glucose level on 0^{th}, 7^{th},14^{th},21^{st} ,30^{th} day of the experiment.

There was significant (P<0.05) blood glucose levels decreased with oral administration of seed extract of *E.robusta* at dose of 50 and 100mg/kg body weight in comparsion to diabetic control. The percentage of glucose reduction was observed at dose 50mg/kg body weight was (41.94%) and 100mg/kg body weight (53.84%) on 30^{th} day.

Hypolipidemic Activity in wistar rats

Table 2: shows the serum lipid profile of the experimental animal on day 0 and day 30 of the study period, no statistically significant difference was noted on 0^{th} day. At the end of the study period *E.robusta* both the dose of 50 and 100mg/kg body weight seed extract treated diabetic animals showed significant hypolipidemic activity. The percentage of reduction in total cholesterol (0.3), serum triglycerides (0.32), serum LDL (1.53), serum VLDL(0.32), and increased the serum HDL (-0.11) significantly (P<0.01) in comparison to the diabetic control group the dose of 100mg/kg body weight.

Table 1: Changes of body weight in control and Diabetic induced rats.

Body weight		
Treatment Groups	**Initial body weight (Mean ± SD)**	**Final body weight (Mean ± SD)**
Normal Control	160.7 ± 1.751	173.7 ± 2.066
Diabetic control	171.4 ± 2.757	168.3 ± 3.502
Standard control (Gliclazide 50mg/kg)	168.2 ± 1.329	180.2 ± 2.944
E.robusta seed extract (T1) 50mg/kg body weight	165.0 ± 1.472	170.2 ± 2.858
E.robusta seed extract (T2) 100mg/kg body weight	176.2 ± 1.862	185.8 ± 2.639

n=6; Data expressed as mean ± SD. Evaluaton by One way Analysis of variance (Anova) Followed by post-hoc tukey's multiple comparison test. P< 0.05; as compared to diabetic control. % of reduction in Initial body weight and final body weight shown in diabetic control -1.80%, standard control 7.13%, Test group T1 3.15%, Test group T2 5.44%.

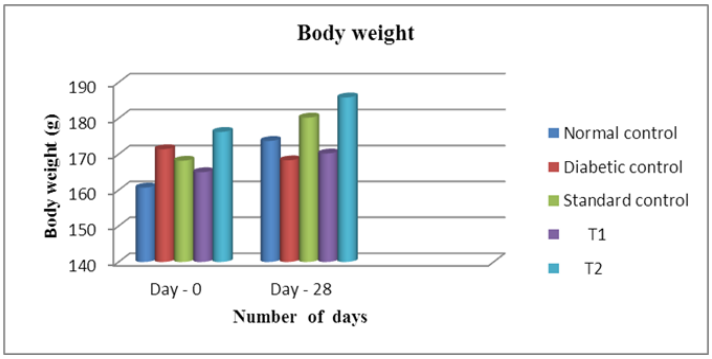

Fig 1: Effect of Methanolic seed extract of *E. robusta* on Body weight in Alloxan induced rats.

Table 2: Effect of Methanolic seed extract of *E.robusta* on blood glucose (mg/dl) level in Alloxan induced diabetic rats.

Blood Glucose (mg/dl)					
Groups	**Day0 (Mean±SD)**	**Day7 (Mean±SD)**	**Day 14 (Mean±SD)**	**Day 21 (Mean±/SD)**	**Day 30 (Mean±/SD)**
Normal control	90.50±5.577	94.33±7.815	91.67±6.212	90.17±3.656	91.33±6.314
Diabetic control	352.3±6.121	355.5±6.058	358.3±4.412	362.2±5.811	371.7±4.033
Standard (Gliclazide) (50mg/kg)	346.3±6.947	272.3±7.005	197.8±5.981	152.8±7.468	131.7±3.559
E.robusta Extract (50mg/kg)	351.7±5.317	331.3±7.118	270.7±7.118	243.7±3.559	204.2±4.021
E.robusta Extract (100mg/kg)	347.7±6.186	273.5±7.662	219.8±6.014	178.0±6.066	160.5±6.025

[Values are mean ± SD from 6 animals in each group]

n=6; Data expressed as mean ± SD. Evaluaton by One way Analysis of variance (Anova) Followed by post-hoc tukey's multiple comparison test. P< 0.05 as compared to diabetic control values <0.05.

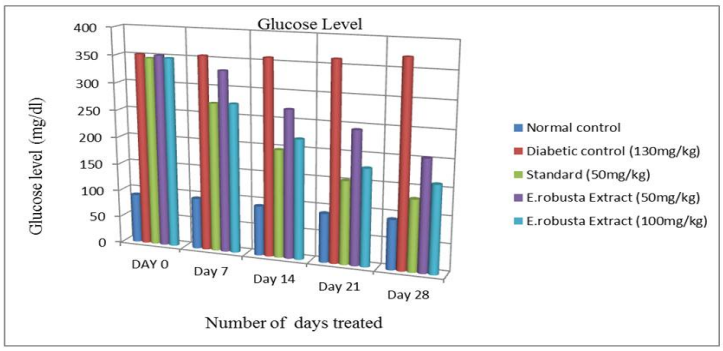

Figure-2.

Table 3: Effect of methonolic seed extract of *E.robusta* on Total Cholesterol and Triglyceride Levels.

Treatment Groups	**Total cholesterol level(mg/dL) (Mean ± SD)**		**Total Triglyceride level(mg/dL) (Mean ± SD)**	
	0-day (Mean ± SD)	**30-day (Mean ± SD)**	**0-day (Mean ± SD)**	**30-day (Mean ± SD)**
Normal Control	80.50± 1.761	81.00 ± 2.280	81.33±2.503	80.50 ± 3.834
Diabetic control	82.67± 3.559	130.2 ± 3.430	84.50±1.871	130.5 ± 2.881
Standard control (Gliclazide 50mg/kg)	81.17± 1.169	110.5 ± 2.168	82.83±2.639	99.50 ± 1.871
E.robusta seed extract (T1) 50mg/kg body weight	84.50± 1.643	126.0 ± 2.608	83.17±2.639	128.2 ± 3.920
E.robusta seed extract (T2) 100mg/kg body weight	83.17±2.858	116.3 ± 3.141	82.00±1.789	108.7 ± 2.582

n=6; Data expressed as mean ± SD. Evaluaton by One way Analysis of variance (Anova) Followed by post-hoc tukey's multiple comparison test. P< 0.05 as compared to diabetic control.

Table 4: Effect of methonolic seed extract of *E.robusta* on HDL, LDL, VLDL Levels.

Treatment Groups	HDL (Mean ± SD)		LDL (Mean ± SD)		VLDL (Mean ± SD)	
	0-day	30-day	0-day	30-day	0-day	30-day
Normal Control	45.17 ± 2.137	45.17 ± 1.722	16.83 ± 2.787	19.73 ± 2.787	16.26 ± 2.805	16.1 ± 1.049
Diabetic control	42.50 ± 2.345	30.00 ± 3.688	21.17 ± 2.994	74.1 ± 3.488	16.9 ± 2.401	26.1 ± 3.141
Standard control (Gliclazide 50mg/kg)	43.50 ± 1.517	40.67 ± 1.506	18.50 ± 1.871	49.93 ± 2.582	16.56 ± 2.251	19.9 ± 1.862
E.robusta seed extract (T1) 50mg/kg body weight	41.00 ± 2.000	33.17 ± 3.869	24.33 ± 2.160	67.73 ± 2.366	16.63 ± 2.366	25.6 ± 1.378
E.robusta seed extract (T2) 100mg/kg body weight	40.17 ± 1.329	35.67 ± 1.751	23.17 ± 1.329	58.63± 1.941	16.4 ± 1.871	21.7 ± 1.414

n=6; Data expressed as mean ± SD. Evaluaton by One way Analysis of variance (Anova) Followed by post-hoc tukey's multiple comparison test. P< 0.05 as compared to diabetic control.

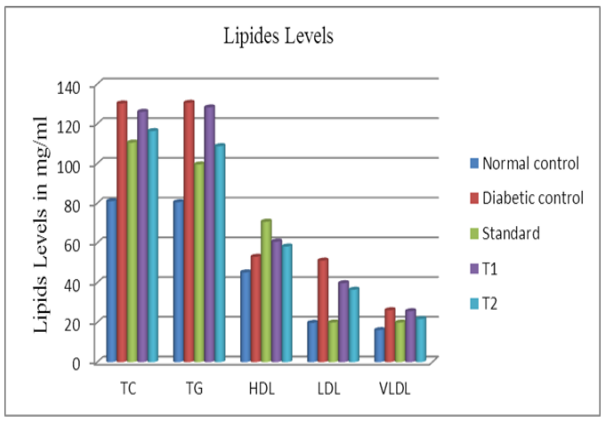

Fig 3: Effect of Methanolic seed extract of *E. robusta* on TC, TG, HDL, LDL, VLDL.

DISCUSSION

Alloxan induces "chemical diabetes" in a wide variety of animal species by damaging the insulin secreting ß-cell of the pancreas.[12] Literature study indicate that alloxan rats are hyperglycemic and hyperlipidemic.[13] The use of lower doses of alloxan (130mg/kg body weight) produced a partial destruction of pancreatic ß-cells and the animals became diabetic.[14]

In this study treatment with methonolic seed extract of *E.robusta* showed significant increase(P<0.05) in body weight at the dose of 50mg/kg and 100mg/kg body weight in Group IV and Group–V in comparison with standard control in Group III.

In Acute oral toxic study results, no lethal/toxic reactions were observed at the dose 50mg/kg & 100mg/kg till the end of the study periodand showed no effect even at the dose of 1000 mg/kg body weight. Similar toxicity study done in flower buds of *Eugenia caryophyllus* at the dose 200mg/kg and 400mg/kg and in bark of *Casuariana equisetifolia* at the dose 400mg/kg body weight did not show any toxicity, It is an indication that the seed extract could be considered relatively safe.[15]

The Oral Glucose tolerance test (OGTT) is commonly used procedure in the diagnosis of diabetes. It measures the body's ability to use glucose. Oral Glucose tolerance test also confirmed blood glucose lowering activity of *E.robusta.* The onset of hypoglycemic action of methonolic seed extract of *E.robusta* at the dose of 50mg/kg body weight was significant (P<0.05) at the period of 0,60,120,180 min showing blood glucose tolerance test values of 92.83 ± 2.483, 168.2 ± 2.563, 118.7 ± 4.033, 116.8 ± 3.061 respectively.

Similarly seed extract of *E.robusta* at the dose of 100mg/kg body weight showed significance (P<0.05) at the period of 0,60,120,180 min showing blood glucose tolerance test values of 83.17±2.858, 116.3 ± 3.141, 82.00±1.789, 108.7 ± 2.582 respectively. Hence it is inferred from above findings that seed extract of *E.robusta* is involved in enhancement of glucose utilization, so blood glucose levels significantly decreased in glucose loaded rats[16] similarly findings were seen in studies done on extracts of *cynoglossum zeylanicum, Barleria cristata, hedyotis DC, Eucalyptus camaldulensis.*

Hypoglycemic activity of seed extract showed significant P<0.05 activity at the dose of 50mg/kg body weight (41.94%) and at the dose 100mg /kg body wt (53.84%). In correlation with the present study earlier *E.robusta* seed extract was found to have Antidiabetic activity at the dose of 100mg/kg and 200mg/kg body weight.[12] Similarly hypoglycemic of plant extract has been reported in many plants like *Berberis aristata*,[17] *Pterocarpus marsupium*,[18] *Allium sativam.*[19]

Hypolipidemic is one of the complication in diabetic mellitus. Insulin has the significant role in lipid metabolism. Insulin deficiency is associated with hypercholesterolemia and hypertriglyceridemia caused by derangement of metabolic abnormalities.[17]

The hypolipidemic activity of methonolic seed extract of *E.robusta* was evaluated in alloxan induced diabetic rats. The diabetic rats (Group-II) showed significant (P<0.05) increase in serum TC (130.2)and TG (130.5) and significantly (P<0.05) decreased HDL (53.00) levelthis is due to the insulin deficiency, high concentration of serum lipids which are increased by lipolysis thus producing more free fatty acid from adipose tissue. Since

insulin has an inhibitory action on HMG-CoA reductase (3-hydroxy-3-methyl-gultaryl coenzyme A reductase), the key enzyme in cholesterol biosynthesis,[18] insulin deficiency or insulin resistance may therefore be responsible for hyperlipidemia. The present study indicated that there was significant (P<0.05) decrease in TC and TG and significant (P<0.05) increased HDL level in alloxan induced diabetic rats after administration of methonolic seed extract of *E.robusta* at the dose 100mg/kg body weight in (Group-IV), comparable to standard control (Gliclazide) at the dose 5mg/kg body weight in diabetic induced rats (Group-III) after 30 days. This study results agree with previous studies of hypolipidemic properties of many Indian medicinal plants and products like *Eclipta prostrata*[19], *Eugenia caryophyllus*,[20] *Erythrina variegate*,[21] *Helicteres isora*[22] which have been reported to be used successfully to manage hyperlipidemia.[17]

CONCLUSION

In summary, methanolic seed extract of *E.robusta* was tested for its hypoglycemic and hypolipidemic activity in alloxan induced diabetic rats. The tested compound showed no lethal effect and proved to be non-toxic even at the dose of 2000 mg/kg body weight. Further, choosen concentrations at the dose of 100mg/kg body weight showed increased body weight. While other parameters like lipid profile, OGTT during different time intervals showed similar effect of decrease in their levels for concentrations of 50 and 100mg/kg body weight. Hence from above findings it can be concluded that this naturally available compound of *E.robusta* has potential to decrease blood glucose and lipid levels exhibiting hypoglycemic and hypolipidemic activity but the exact role of it needs further clarification by Isolating and identifying the active compound to explain the possible mechanism of action of *E.robusta* seed extract.

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REFERNCES

1. Grundy S M. Cholesterol and coronary heart disease: a new era. J Am Med Assoc, 1986; 256: 2849-2858.
2. Davey Smith G. Cholesterol lowering and mortality: the importance of considering initial level of risk. Int. Med. J., 1993; 306: 1367-1373, Correction: 1648.
3. Kaesancini AY and Krauss RM. Cardiovascular disease and hyperlipidemia: Current topics of lipid dynamics, 1994; 5: 249-251.
4. Betteridge. Lipid disorders in diabetes mellitus, in: JC Pickup, G Williams, editor. Text Book of Diabetes. 2nd. London: Blackwell Science, 1997; 55: 1-55.31.
5. Speight TM. In: Avery's Drug treatment Principles and Practice of clinical Pharmacology and therapeutics. 3rd Edition, ADIS press Ltd, 1987; 599.
6. Marles JR, Farnsworth NR. Antidiabetic plants and their active constituents. *Phytomedicine*, 1995; 2(2): 123-129.
7. Loew D, kaszkin M. Approaching the problem of bioequivalence of herbal medicinal products. Phytother Res., 2002; 16: 705-711.
8. Shelar R, Maurya C, Tekale P, Katkar K, Naik V, Suthar A, Chauhan V.S. Embelin – An HPLC Method for Quantitative Estimation in *Embelia ribes* Burm.F. International Journal of Pharmaceutical and Clinical Research, 2009; 1(3): 146-149.
9. Le Floch JP, Escuyer P, Baudin E, Baudon D, Perlemuter L. Blood glucose area under curve. Methodological aspects. Diabetes Care, Feb, 1990; 13(2): 172-5.
10. Tinder P. Determination of blood glucose using an oxidase peroxidise system with a non-carcinogenic chromogen. Ann. Clini. Biochem, 1969; 6: 24-7.
11. Best CH, Taylor NB. Biological effects of insulin. In: Williams W, Wilkins, editors. Physiological basis of medical practice. London: Cambridge University Press, 1989.
12. Bopanna KN. Anti diabetic and antihyperlipidemic alloxan diabetic rabbits. Indian J Pharmacol, 1997; 29: 162-7.
13. Prince SM, Menon VP.Hypoglycemic and other related actions of *Tinospora cardifolia* roots in alloxan induced diabetic rats. J Ethanopharmacol, 2007; 70: 9-15.
14. Cooke DW, Plotnick L. "Type 1 diabetes mellitus in pediatrics". Pediatr Rev. November 2008; 29(11): 374–84. quiz 385. doi:10.1542/pir.29-11-374. PMID 18977856.
15. S.K. Prasad, Alka Kulshreshtha and Taj. Qureshi, Antidiabetic avtivity of Some Herbal Plants in Streptozotocin Induced Diabetic Albino Rats. Pakisthan Journal of Nutrition, 2009; 8(5): 551-557.
16. R.Narmadha, K.Devaki, Toxicological evalution and oral glucose tolerance test of ethonolic leaf extract of *Barleria cristata* L. in wistar rats. International journal of Basic & Clinical Pharmacology, 2013.
17. P.Mishra, A.Rani and P.Mitra Mazumder, Blood Glucose Lowering Potential of stem of *Berber aristata* DC in Alloxan –Induced Diabetic Rats, *Iranian Journal of Pharmacology & Therapeutics*, 2010; IJPT 9: 21-24.
18. A.Maruthupandian and V.R.Mohan, Antidiabetic, Antiyperlipidaemic and Antioxidant activity of *Pterocarpous Marsupium* Roxb in alloxan induced diabetic rats. *International Jounrnal of Pharma Tech Research*, July-Sept, 2011; 3(3): 1681-1687.
19. Poonam Tripathi, Prem Prakash Gupta, Vijay Kumar Lal, Effect of Co-administration of *Allium sativum* extract and Metformin on blood glucose of

Streptozotocin induced diabetic rats. *J Intercult Ethnopharma col*., 2013; 2(2): 81-84.

20. Pankaj G, Jain, Sanjay J, Surana, A review of Indian medicinal plants with hypolipidemic activity and their medicinal importance. World Journal of Pharmacy and Pharmaceutical sciences, 4(03): 1477-1493.
21. Kumari CS, Govindasamy S, Sukumar E. Lipid lowering activity of *Eclipta prostrate* in experimental hyperlipidemia. J. Ethnopharmcol, 2006; 105: 332-5.
22. Roma Ghai, K.Nagarajan, Vinay Kumar, Parul Grover, Antihyperlipidemic potential of *Eugenia caryophyllus* extract in Wistar rats. International journal of phytomedicine, 2014; 6: 412-416.
23. G.Balamuragan, A.Shantha, Effect of *Erythrina variegate* seed extract on hyperlipidemia elicited by high-fat diet in wistar rats. J Pharm Bioallied Sci., Oct-Dec, 2010; 2(4): 350-355.
24. Boopathy Raja A, Elanchezhiyan C, Sethupathy S. Antihyperlipidemic activity of *Helicteres isora* fruit extract on streptozotocin induced diabetic male wistar rats. Eur Rev Med Pharmacol Sci., Mar, 2010; 14(3): 191-6.
25. Venu Gopala rao konda, madhavi. E, Ruckmani.A and Venkataramana. Y. A review on medicinal plants with potential with hypolipidemic activity. Int J Pharm Bio Sci., Oct, 2013; 4(4): 729-740.