



ANTI DIABETIC POTENTIAL OF ETHANOLIC EXTRACT OF SEED KERNEL OF *SAPINDUS EMARGINATUS* IN ALLOXAN AND DEXAMETHASONE INDUCED DIABETIC RATS

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ABSTRACT

The objective of the study was to evaluate the Anti diabetic potential of ethanolic extract of seed kernel of *Sapindus emarginatus* in Alloxan induced diabetic rats. Toxicity study of the ethanolic extract of *Sapindus emarginatus* seed kernel (EESESK) was carried out in Albino mice up to a dose limit of 2000 mg/kg orally. The screening of antidiabetic activity was done in two selected models for our study. Alloxan hydrate at a dose of 150 mg/kg i.p after 48 hrs, the EESESK seed kernel at a dose of 200 mg/kg and 400 mg/kg p.o were administered. Dexamethasone 10 mg/kg, i.p once daily and at the interval of 30 mins EESESK 200 mg/kg and 400 mg/kg p.o were administered in rats for a period of 11 and 21 days respectively. At the end of the experimental period, serum biochemical parameters like glucose, total cholesterol, triglycerides, LDL, HDL, and VLDL were analysed by GOD-POD method using semi autoanalyser. In both models EESESK at dose of 400 mg/kg showed significant effect. The present study indicates that *S.emarginatus* seed kernel antidiabetic agent useful for the management of diabetes mellites. *S.emarginatus* seed kernel showed significant reduction of Glucose TC, TG, LDL, VLDL levels and increased level of HDL in diabetic model rats and the increased HDL (cardioprotective lipid). Therefore *S.emarginatus* seed kernel has potential role to prevent formation of atherosclerosis and coronary heart disease.

Keywords: *Sapindus emarginatus*, Alloxan and Dexamethasone induced methods

INTRODUCTION

Diabetes is a metabolic disorder across the Globe. It is characterized by constant high levels of blood glucose (sugar)¹. Human body has to maintain the blood glucose level at a very narrow range, which is done with insulin and glucagon. Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine². Insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus³. Diabetes has emerged as major problem according to Indian national Diabetes Federation (IDF); there

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore, it was estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025. The countries with the largest number of diabetic people will be India, China and USA by 2030⁴. It is estimated that every fifth person with diabetes will be an Indian. Due to these sheer numbers, the economic burden due to diabetes in India is amongst the highest in the world⁵. The real burden of the disease is however due to its associated complications which lead to increased morbidity and mortality.

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The classic symptoms of untreated diabetes are loss of weight, polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger)⁶⁻⁹.

MATERIAL AND METHODS

Collection of Soap nut seed kernel

The soap nuts were collected from the local market of jagannadhapuram in Andhra Pradesh (India) during the month of January, 2013.

Extraction procedure

Soap nut seed kernels were subjected to drying in normal environmental conditions under shade. The dried seed kernels were powdered with the help of a hand mill and were stored in air tight container. The powdered material was passed through sieve of 16 mesh size to obtain uniform particle size for extraction. 500 g of powder was defatted with petroleum ether at 50-60°C for 24 hr. After 24 hr the dry defatted powder was poured into the soxhlet apparatus. Sufficient solvent (90% ethanol) was added into the flask and the soxhlet apparatus was placed on the mantle along with 3-4 ceramic chips. The flask was fitted with a water-cooled condenser¹⁰⁻¹⁴. The mantle was switched on and the temperature was set at 45°C. The extraction was continued for 36 hr, 1-2 cycles per hour⁴. After 36 hr the mantle was switched off and water flow was stopped. After cooling the plant material was removed by filtration through a cotton plug. The solvent of the extract was evaporated by rotatory flask evaporator. The extract mass was weighed in a digital balance. The extract was labeled and kept in desicator for further use.



Figure 1: *Sapindus emarginatus* seed kernel

Preliminary Phytochemical screening

The methanolic extract obtained from the above extraction processes was analyzed for different phytoconstituents present in it by the standard qualitative phytochemical analysis methods. The following tests revealed the presence of different chemical constituents such as flavonoids, saponins, steroids, glycosides²⁵.

Experimental design

Male and Female albino Sprague Dawley rats 7-8 weeks old and weighing approximately 150-250 g were randomly used for the present study. The animals were obtained from Bapatla College of Pharmacy from Bapatla. The animals were maintained under control condition with 12 h light and 12 h dark cycles at temperature 24±1°C and humidity 55±5%. The animals were randomised into control and experimental groups and housed in 8 cages (Six animals in each cage). All rats were provided with food and water ad libitum during the experiment¹⁵⁻¹⁹. The control and experimental animals were provided food and drinking water *ad libitum*. All animals were acclimatized for minimum period of 1 week prior to the beginning of study. The study was performed according to the Indian National Science Academy Guidelines for the care and use of animals in scientific research.

Toxicity studies

Toxicological studies were done according to the guidelines of Organization for Economic Cooperation & Development (OECD no.423). Initially, the dose was administered to single female rat and the rat observed for 48 hrs, with close surveillance up to initial 4 hrs. After 48 hrs (of the first administration) same dose was administered in 2 more female rats and the were observed for 48 hrs with close surveillance upto initial 4hrs (same as in case of first rat). After 48 hrs (of second administration), same dose was administered in 2 more female rats and observation was done same as for previous rats²⁰⁻²⁴. The rats were observed for 14 days for any toxic reaction. So, 1/10th, 1/20th doses from the studies was selected for the present experimental study to check the effect of antidiabetic activity of *Sapindus emarginatus*.

Table 1: List of chemicals used in experiment

S. No	CHEMICALS	COMPANY
1	Alloxan hydrate (25gm)	National scientific Pvt Ltd, Mumbai, India. Mfg Lic no: G/2407-
2	Dexamethasone	A,Cadila Health care Limited, India. Mfg Lic no:G/811, Sanofi
3	Glibenclamide	India Limited, India. Merck specialities Pvt Limited, Mumbai, India.
4	Tween 80	

EXPERIMENTAL PROCEDURES

1. Alloxan induced diabetes in rats:

Group I: Normal saline

Group II: Animals received Alloxan hydrate at a dose 150 mg/kg i.p

Group III: Animals received alloxan hydrate at a dose of 150 mg/kg i.p after 48 hrs, the Glibenclamide at a dose of 5 mg/kg p.o were administered.

Group IV: Animals received Alloxan hydrate at a dose of 150 mg/kg i.p after 48 hrs, the EESE seed kernel at a dose of 200 mg/kg p.o were administered.

Group V: Animals received Alloxan hydrate at a dose of 150 mg/kg i.p after 48 hrs, the EESE seed kernel at a dose of 400 mg/kg p.o were administered.

2. Dexamethasone induced diabetes in rats:

Group I: Normal saline

Group II: Animals received Dexamethasone at a dose 10 mg/kg i.p

Group III: Animals received Dexamethasone at a dose of 10 mg/kg i.p and after 30 mins, the Glibenclamide at a dose of 5 mg/kg p.o were administered.

Group IV: Animals received Dexamethasone at a dose of 10 mg/kg i.p and after 30 mins, the EESE seed kernel at a dose of 200 mg/kg p.o were administered.

Group V: Animals received Dexamethasone at a dose of 10 mg/kg i.p and after 30 mins, the EESE seed kernel at a dose of 400 mg/kg p.o were Administered.

Blood sample collection method

The tip of the capillary was inserted at the medical canthus into the retro-orbital

plexus. Capillary tube: 1mm (bore size). The animal was restrained (unanaesthetised) in such a way that loose skin of the neck was tightened with gentle rotation by the other hand as the vessels are ruptured, blood wells up in the peri-orbital space²⁶⁻²⁷. The tip of the capillary was then slightly withdrawn, so that the blood flows into the capillary, which was collected in microcentrifuge (1ml) tube containing anticoagulant for biochemical estimations.

Statistical analysis

Statistical analysis was calculated by T-paired test. Mean and SEM VALUES were calculated.

RESULTS

Alloxan induced diabetes in rats

Glucose levels

Normal groups were administered saline and blood glucose levels are found to be 66.66 mg/dL to 69 mg/dL of glucose levels throughout the experiment. Toxicant group was administered with alloxan hydrate at a dose of 150 mg/kg and blood glucose levels are found to be from range of 275 mg/dL to 303 mg/dL. Standard group of animals received glibenclamide at a dose of 5 mg/kg & is able to reduce blood glucose levels to 125 mg/dL against alloxan induced diabetes in rats. The animals which received ethanolic extract of *EESESK* at a dose of 200 mg/kg has reduced blood glucose levels and it found to be 192 mg/dL, 171.79 mg/dL, 162.23 mg/dL on 11th, 14th and 21st days of the experiment. The animals which received ethanolic extract of *EESESK* at a dose of 400 mg/kg has reduced blood glucose levels and it found to be 290.76, 243.33, 139.16mg/dL on 11th, 14th and 21st days of the experiment against Alloxan induced Diabetes in Rats.

Lipid profile

The control group of rats exhibited normal lipid levels i.e total cholesterol (66.35), triglycerides (68.22 mg/dL), LDL (35.29 mg/dL), VLDL (13.64 mg/dL), and HDL (85.75 mg/dL). Alloxan treated rats had shown increased lipid levels TC, TG, LDL, VLDL, and reduced HDL levels was found to be 200 mg/dL, 393 mg/dL, 87.01 mg/dL, 79 mg/dL, and 33.99 mg/dL respectively. Standard group

of animals received Glibenclamide at a dose of 5mg/kg reduced lipid levels to TC (111.6 mg/dL), TG (102.75 mg/dL), LDL (32.68 mg/dL), VLDL (20.55mg/dL), and increased HDL levels (58.36 mg/dL) was found when compared to toxicant group. The animals which received EESM at a dose of 200 mg/dl as reduced lipid levels of TC (99.69mg/dl), TG(137.19mg/dl), LDL(27.43mg/dl),

VLDL(27.43) VLDL(27.43) and increased HDL levels (40.75 mg/dL), when compared to toxicant group. The animals which received EESM at a dose of 400mg/dl as reduced lipid levels TC (74.75 mg/dl), TG(90.02 mg/dl), LDL(25.25 mg/dl), VLDL(18 mg/dl) and increased HDL(77 mg/dl) was found when compared to toxicant group.

Table 2: Effect of ethanolic extract of seed kernel of *S. emarginatus* on Blood glucose levels

Days	Normal	Toxicant	Standard	200mg	400mg
0	66.66±2.390	303±2.23	306.9±0.22	260.6±2.24	418±2.23
7	66.33±2.27**	294.9±2.23	295.5±2.46ns	192.2±2.23**	290.76±2.10**
14	69±2.56**	285±2.23	240±2.58**	171.79±1.66**	243.33±2.10**
21	67.72±2.39**	275±2.26	125±2.23**	162.23±3.82**	139.16±3**

Significance at * p>0.05, ** p>0.01, *** p>0.001, ns = no significant at n = 6

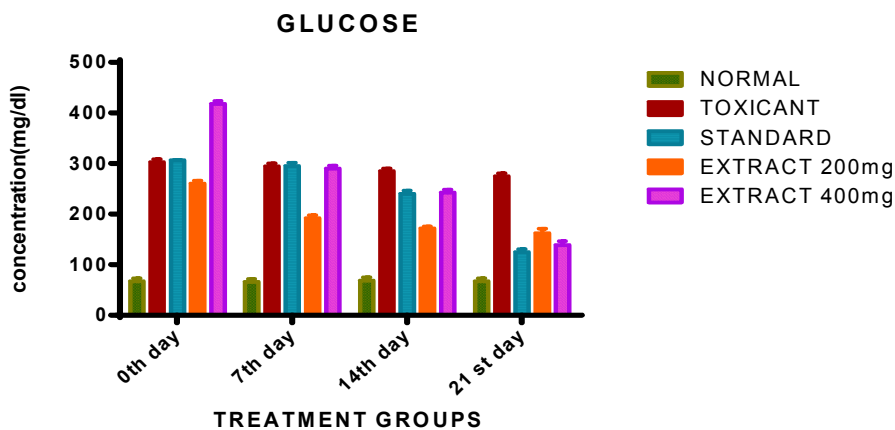


Figure 2: Effect of ethanolic extract of seed kernel of *S. emarginatus* on Blood glucose levels

Table 3: Effect of ethanolic extract of seed kernel of *S. emarginatus* on lipid levels

S. No	Treatment Groups	TC (mg/dL)	TG (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)
1	Normal	66.35±2.23	68.22±2.23	35.29±1.34	13.640±0.44	85.75±0.33
2	Toxicant	200±2.23	393.33±3.33	87.01±0.44	79±0.44	33.99±2.23
3	Standard	111.6±2.23**	102.75±2.23**	32.68±0.28**	20.550±0.44**	58.36±1.50**
4	Extract 200mg	99.69±2.97**	137.190±2.23**	29.43±2.89**	27.430±0.44**	40.75±0.33**
5	Extract 400mg	74.75±2.105**	90.02±2.23**	25.53±0.43**	18±0.44**	77±0.89**

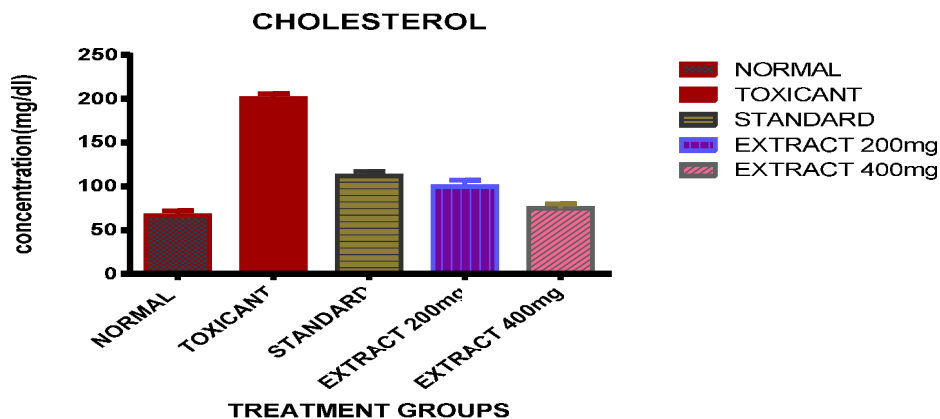


Figure 3: Effect of ethanolic extract of seed kernel of *S. emarginatus* on lipid levels (cholesterol)

Table 4: Effect of eth. Extract of seed kernel of *S.emarginatus* on alloxan induced diabetic rats on body weight

S. No	GROUPS	BODY WEIGHT (gm)	
		0 th day	21 st day
1	Normal	146.66±2.10	165.83±3.0
2	Toxicant	222.50±1.1	202.50±1.18
3	Standard	167.50±3.35	174.66±3.2
4	EXTRACT (200mg)	177.50±1.11	184.5±2.3
5	EXTRACT (400mg)	181.66±3.8	188.83±4.2

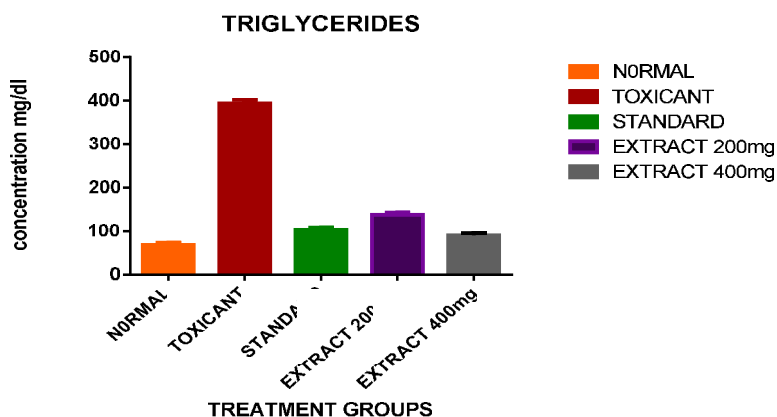


Figure 4: Effect of ethanolic extract of seed kernel of *S. emarginatus* on lipid levels (triglycerides)

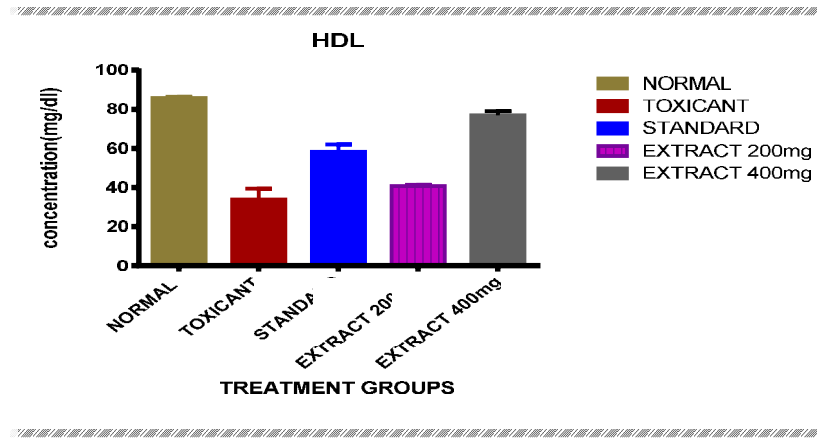


Figure 5: Effect of ethanolic extract of seed kernel of *S. emarginatus* on lipid levels (HDL)

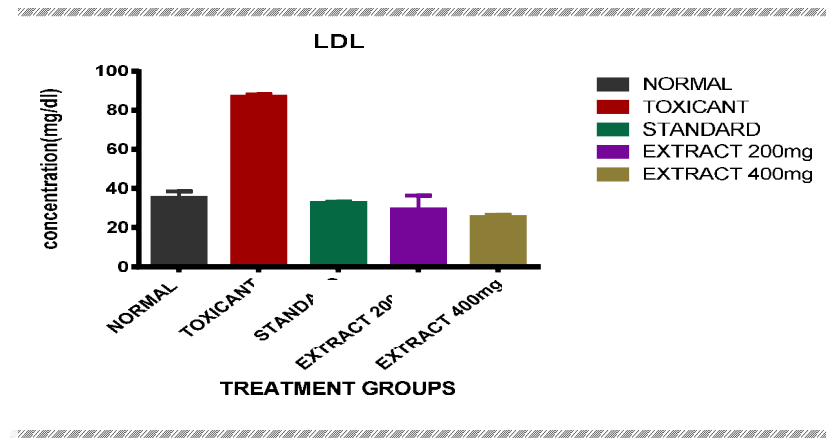


Figure 6: Effect of ethanolic extract of seed kernel of *S. emarginatus* on lipid levels(LDL)

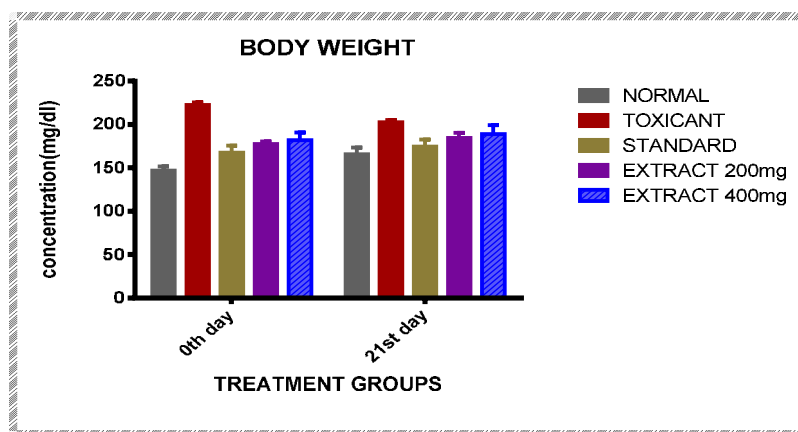


Figure 7: Effect of eth. Extract of seed kernel of *S.emarginatus* on alloxan induced diabetic rats on body weight

DEXAMETHASONE INDUCED DIABETES

Glucose levels

A control group was administered saline and glucose levels found to be 77.91 mg/dL to 78.34 mg/dL of throughout the experiment in blood. Toxicant groups were administered with Dexamethasone at a dose of 10mg/kg and blood glucose levels are found to be 206.18 mg/dl.

Standard group of animals received Dexamethasone (10mg/kg) +Glibenclamide (5 mg/kg) has shown reduced glucose levels from 206.18 mg/dL to 78.20 mg/dL. The animals which received Dexamethasone (10mg/kg) +EESM at a dose of 200 mg/kg has reduced blood glucose levels from 206.18 mg/dL to 148.54 mg/dL.

The animals which received Dexamethasone (10mg/kg) +EESM at a dose of 400 mg/kg have reduced blood glucose levels from 206.18 mg/dL to 90.08 mg/dL.

Lipid profile

The control group of rats exhibited normal lipid levels of TC and HDL& was found to be 70.85 mg/Dl and 66.60 mg/dL respectively. Dexamethasone treated rats has shown increased levels of TC(170.40 mg/dL) and reduced HDL levels of (29.6 mg/dL) when compared to control groups. Standard group of animals received Dexamethasone (10 mg/kg) + Glibenclamide (5mg/kg) as reduced cholesterol levels (100.31 mg/dl) and increased HDL levels (56.69 mg/dl) when compared to toxicant group. The animals which received Dexamethasone (10 mg/kg) + EESM at a dose of 200 mg/kg has exhibited decreased levels of TC (116 mg/dL), and increased levels of HDL (62.04 mg/dL) when compared to toxicant group. The animals which received Dexamethasone (10 mg/kg) + EESM at a dose of 400 mg/kg has reduced TC levels (70.81 mg/dL), and increased HDL levels (69.06 mg/dL) when compared to toxicant groups.

BIOCHEMICAL PARAMETERS

Table 5: Effect of ethanolic extract of seed kernel of *S. emarginatus* on blood glucose levels.

DAYS	NORMAL	TOXICANT	STANDARD	200mg	400mg
0	77.91±3.64	65.92±1.95	69.07±2.52	68.33±2.84	66.85±2.42
11	78.34±3.56**	206.18±0.42	78.20±3.76**	148.54±3.82**	90.08±12.73**

Figure 8: Effect of ethanolic. Extract of seed kernel of *S. emarginatus* on blood glucose levels

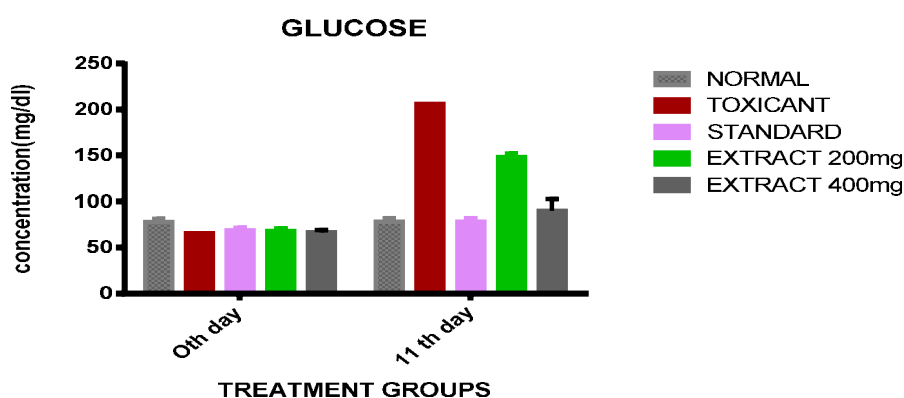


Table 6: Effect of Ethanolic extract of seed kernel of *S. emarginatus* (EESG) on lipid levels

S. No	GROUP	DOSE	Total cholesterol (mg/dL)	HDL (mg/dL)
1	Normal control	NORMA SALINE	70.85±3.29	66.60±0.11
2	Diabetic control	10g/kg	170.40±4.29	29.60±0.11
3	Standard	5mg/kg	100.31±2.05**	56.69±0.008**
4	Ethanolic extract	200mg	116±4.67**	62.04±0.006**
5	Ethanolic extract	400mg/kg	70.81±0.46**	69.06±0.08**

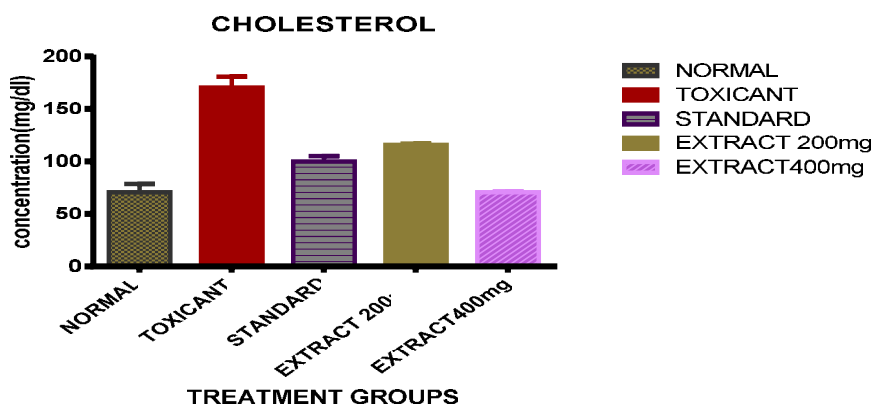


Figure 9: Effect of Ethanolic extract of seed kernel of *S. emarginatus* (EESG) on lipid levels (cholesterol)

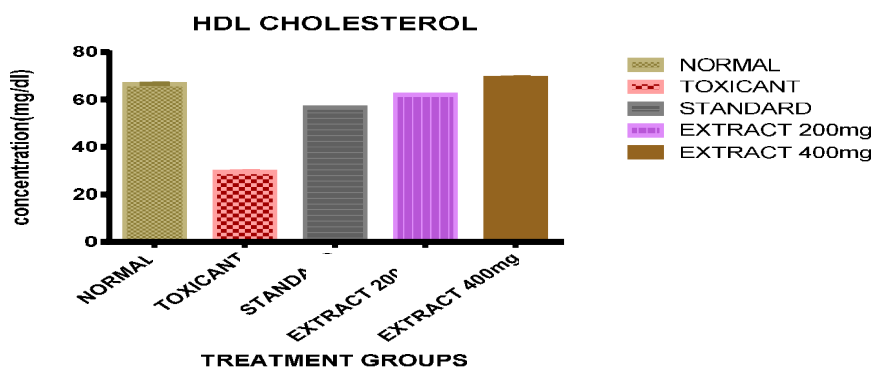


Figure 10: Effect of Ethanolic extract of seed kernel of *S. emarginatus* (EESG) on lipid levels (HDL)

S. No	GROUPS	BODY WEIGHT (gm)	
		0 th day	11 th day
1	Normal	151.50±0.671	172.66±2.10
2	Toxicant	163.33±1.05	211.66±1.66
3	Standard	156.66±1.66	129.16±1.53
4	EXTRACT (200mg)	159.16±2.38	134.16±0.83
5	EXTRACT (400mg)	179.16±3.51	151.66±1.05

Table 7: Effect of eth. Extract of seed kernel of *S. emarginatus* on Dexamethasone induced diabetic rats on body weight

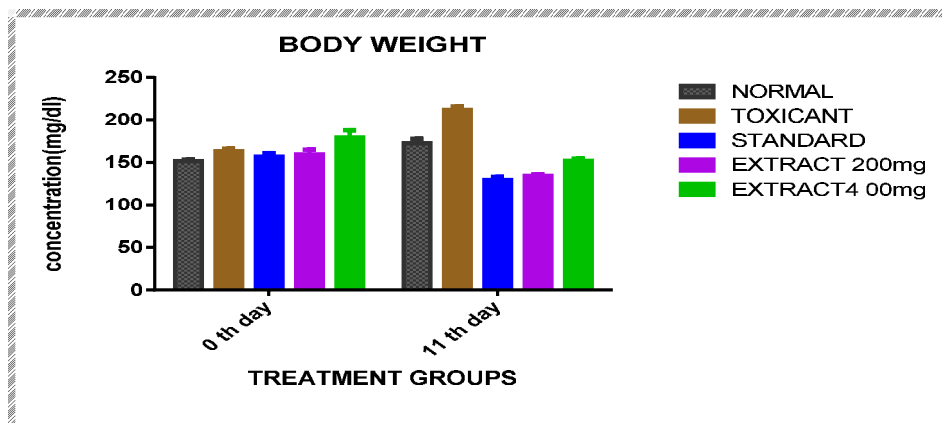


Figure 11: Effect of Ethanolic Extract of seed kernel of *S. emarginatus* on Dexamethasone induced diabetic rats on body weight

DISCUSSION

The high doses of ethanolic seed kernel extract of *Sapindus emarginatus* reduced the blood glucose of the experimentally induced diabetic rats. There was also a decrease in the levels of serum cholesterol, triglycerides, low density lipoproteins and very low density lipoproteins, while high density lipoproteins increased²⁸⁻³⁰. These suggested that the ethanolic seed kernel extract of *S.emarginatus* had antidiabetic activity. The levels of cholesterol, triglycerides, low density lipoproteins and very low density lipoproteins are generally increased in diabetic condition. Insulin play a role metabolism of carbohydrates and lipids, it acts as a potent inhibitor of lipolysis inhibiting the activity of the hormone sensitive lipase in adipose tissues and the release of free fatty acids³¹.

Diabetic condition leads to enhance the activity of lipase increase lipolysis and releases more fatty acids into blood circulation. Increased fatty acids concentration also increases the β -oxidation of fatty acids produces more acetyl CoA enzyme and cholesterol during diabetic condition. The HDL cholesterol acts as scavenger of cholesterol from the tissues and their by decreases excess cholesterol levels and this leads to fall in serum cholesterol levels.

The diabetes remained as incurable and most complicated pathological condition which affects different vital organs in human body. Based on the ayurveda test *S.emarginatus* has been chosen to evaluate antidiabetic activity. The extract was prepared with 90% of alcohol by continuous percolation process. The alcoholic extract of seed kernel of *S.emarginatus* was subjected for oral acute

toxicity studies according to OECD guidelines No.423 and was found no mortality. Alloxan destroy β cells of islets of langerhans of pancrease and reduces synthesis and release of insulin this condition called diabetes. In Alloxan diabetes rats the blood glucose levels were in the range of 275 to 317 mg/dL which were considered as severe diabetes³². In the standard drug Glibenclamide (5 mg/kg), ethanolic extract (200 mg/kg) and (400 mg/kg) treated groups, the peak values of blood sugar significance decreased to 134.95 mg/dl, 158.86 mg/ dl and 126.95 mg/dL on the 21st day respectively.

The ethanolic extract (400 mg/kg) was found to be almost significant as standard drug in lowering blood glucose levels and also lowers the lipid profile TC (75.26 mg/dL), TG (89.81 mg /dL), LDL (25.51 mg/dL), VLDL (17.95 mg/dL) and increase HDL (75.94 mg/dL) cholesterol levels.

Dexamethasone is a potent and highly selective glucocorticoid used in the treatment of inflammation. Glucocorticoids induced hyperglycemia due to increased hepatic glucose production and insulin resistance o peripheral tissues. The mechanism involves increased α_2 -adrenoreceptor signaling, increased potassium channel activity and impaired glucose metabolism. Dexamethasone induces peripheral insulin resistance and there by inhibiting GLUT-4 translocation, increasing lipase activity in adipose tissue thereby causing impairment of endothelium-dependent vasodilation. Dexamethasone increases the triglycerides levels causing an imbalance in lipid metabolism leading to hyperlipidemia and increases glucose levels leading to hyperglycemia³³⁻³⁴.

Pharmacological doses of glucocorticoids induces ob gene expression in rat adipose tissue within 24 hrs and is followed by a complex metabolic changes resulting in decrease in food consumption causing reduction in body weight accompanied by diabetes and development of Insulin resistance with enhanced glucose and triglycerides levels. In the present study administration of Dexamethasone for 11 days resulted in increased glucose, total cholesterol, and decreased HDL levels. *S.emarginatus* at a dose of 200 and 400 mg/kg significantly prevented the rise in glucose and lowers the total cholesterol and increase HDL cholesterol levels³⁵.

CONCLUSION

The present study indicates that *S. emarginatus* seed kernel was antidiabetic agent useful for the management of diabetes mellites. *S.emarginatus* seed kernel showed significant reduction of Glucose, TC, TG, LDL, VLDL levels and increased level of HDL in diabetic model rats. Therefore *S.emarginatus* seed kernel has potential role to prevent formation of atherosclerosis and coronary heart disease.

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