

ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *SAPINDUS EMARGINATUS* LEAVES

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ABSTRACT

The pharmacological and acute toxicity studies of ethanolic extract of *Sapindus Emarginatus* Leaves was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed up to 2000mg/kg of body weight. The Biological dose of extract *Sapindus Emarginatus* dose was selected 200mg/kg and 400mg/kg in this dose possessed significant antidiabetic activity. In conclusion, in the present study on the ethanolic extract of *Sapindus Emarginatus* leaves having antidiabetic activity more over nearest activity of *Glibenclamide*. This study shows that flavanoids present in this extract may be possibly responsible for the antidiabetic activities. Further pharmacological and biochemical investigation are to be done to find out the active constituent responsible for the antidiabetic activity. However, the future study may also include cataloging, standardizing, for quality control and above all developing new drugs/ pharmaceuticals keeping the disease and cost factor in view.

KEYWORDS: *Sapindus Emarginatus*, acute toxicity, ethanolic extract, *Glibenclamide*.

INTRODUCTION

Herbal medication is the utilization of plants, plant parts, their water or dissolvable concentrates, fundamental oils, gums, pitches, exudates or other type of cutting edge items produced using plant parts utilized restoratively to give proactive help of different physiological frameworks; or, in a progressively customary medicinal sense, to treat, fix, or avert an ailment in creatures or people (Weiss RF and Fintelmann V. et al, 2000).

As per WHO, 78-80% of the population rely on non-regular prescription in their essential health services (Akerlele O et al., 1993). Current centuries, there has been a growing interest in alternative treatments and the satisfying use of natural products, especially those derived from plants (Vulto AG & Smet PAGM et al, 1988). This interest in medications of plant derivation is due to several reasons, namely, conventional drug can be ineffective (e.g. side effects & ineffective treatment), abusive and/or incorrect use of synthetic medicines results in side effects and other complications, a percentage of the world, conventional pharmacological treatment, and folk medicine and natural awareness suggest that "natural" products are harmless.

About 25% of the medications recommended worldwide wide originate from plants, 121 such dynamic mixes being in current use. Of the 252 medications considered

fundamental and basic by the World Health Organization (WHO, 1991), 11% are solely of the plant's birthplace and a noteworthy number are manufactured medications obtained from characteristic antecedents.

It is a local animal types developed in Indo-Gangetic fields, Shivaliks and Sub-Himalayan tracts in India at elevations ranges from 200m to 1500m.^[3] It is referred to locally as cleanser nut in English, Ritha in Bengali and Ponnangottai in Tamil.^[4] The plant contains tetra cyclic tri terpenoid saponins, bacosides An and B, hersaponin, alkaloids viz herperstine what's more, brahmine and flavonoides.^[7,8] All pieces of the *Sapindus emarginatus* contain phenolic acids, for example, protocatechuic corrosive, cis-p-coumaric corrosive, p-hydrobenzoic corrosive and Cinnamic corrosive.^[9] It having various Pharmacological activities Antihyperglycemic and antidiabetic activity, Antifertility and antiandrogenic activity, Antihyperlipidemic activity etc.



Fig: Leaf of Sapindus Emarginatus.

MATERIALS AND METHODS COLLECTION AND IDENTIFICATION

Collection of specimen

The species for the proposed study that is leaves of sapindus emarginatus collected carefully from the Vengamukkalapalem, Pondur Road, Ongole, Prakasam, AP.

Shade drying

After gathering, the leaves of we Sapindus emarginatus rewashed altogether with water to expel the earth particles and subterranean insect other outside material holds fast to leaves. At that point after, the leaves were cleared off with cotton fabric and moved to paper and equally spreader on to paper. The sapindus emarginatus leaves were exposed to shade drying to treat growth until complete dryness of leaves. At that point the dried leaves were powdered by blender processor until to get coarse powder, which was utilized for additionally nitty gritty investigations, extraction with dissolvable and phytochemical ponders.

Toxicological Evaluation

Determination of LD₅₀ value of ethanolic extract of Sapindus Emarginatus Acute Oral Toxicity Study:

The strategy was trailed by utilizing OECD rules 423 (Acute harmful class technique). The intense poisonous class strategy is a stage shrewd system with 3 creatures of single sex per step. Contingent upon the mortality and/or doomed status of the creatures, by and large 2-4 stages might be important to permit judgment on the intense poisonous quality of the guinea pigs while taking into account satisfactory information based logical end. The technique uses characterized dosages (5, 50, 300, 2000mg/kg body weight) and the outcomes enable a substance to be positioned and arranged by the Globally Harmonized System (GHS) for the characterization of compound which causes intense danger.

Animals

Female pale skinned person mice gauging 20-25g were utilized in the present examination. All rodents were kept at room temperature of 22-25°C in the creature house.

Every one of the creatures pursued the universally acknowledged moral rules for the consideration of research facility creatures. Preceding the investigations, rodents were sustained with standard nourishment for multiple weeks so as to adjust to the research facility conditions.

Procedure

Twelve creatures Albino mice, (25-30gm) were chosen for their studies. The beginning portion of ethanolic concentrates of sapindus emarginatus 300mg/kg, b.w, p.o, was managed. The vast majority of the rough concentrates have LD₅₀, esteem more than 2000mg/kg of the body weight of the creature utilized. Portion volume was regulated 0.1ml/100gm body weight to the creature by oral course. In the wake of offering the portion harmful hints were seen inside 3-4 hours. Body weight of the creatures when organization, beginning of harmfulness and indications of poisonous quality like changes in the skin and hide, eyes and mucous layer and furthermore respiratory, circulatory, autonomic and focal sensory systems and somatomotor action and personal conduct standard, indication of tremors, spasm, salivation, loose bowels, torpidity and rest and extreme lethargies was likewise to be noted, assuming any, was watched. The creature dangerous or passing, was seen for as long as 14 days.

OBSERVATION

Intense danger studies and assessment of dates are considered according to the rule of OECD (423). No danger or passing was watched for these given portion levels, in chosen and treated creatures. So the LD₅₀ of the ethanolic concentrate of leaves of Sapindus emarginatus was more prominent than 2000mg/kg (LD₅₀>2000mg/kg). Subsequently the natural portion was fixed at three levels, 200 and 400mg/kg body weight for the concentrate.

Pharmacological Evaluation

The medium-term fasted (18hr) typical rodents were taken and isolated into four gatherings comprises of six creatures. They were furnished with drinking water as it were. Typical saline arrangement was directed to assemble I creatures. Gathering II creatures were gotten Glibenclamide (3mg/kg, b.w) as a standard Sapindus emarginatus separate (200 and 400 mg/kg) was controlled by oral course to amass III and IV Glucose (2mg/kg) load was encouraged 30 minutes after the organization of concentrates. Blood was pulled back from tail vein under gentle ether anesthesia beginning, 30, 60 and an hour and a half after glucose organization (V. Babu et al., 2003) and glucose level were evaluated utilizing glucose strips and a glucometer (Standard diagnostics Ltd). Blood glucose levels were noted and announced.

Evaluation of Anti-Diabetic Activity

Animals

Female pale skinned person Wistar rodents weighing 150-

250g were utilized in the present investigation. All rodents were kept at room temperature of 22-25°C in the creature house. Every one of the creatures were pursued the globally acknowledged moral rules for the consideration of research facility creatures. Preceding the tests, rodents were bolstered with standard sustenance for multi week so as to adjust to the lab conditions as per the proposals for the correct consideration and utilization of research facility creatures.

Chemicals

Alloxan monohydrate (LOBA Chemie, Mumbai, India) was obtained, protected at 25°C and utilized for this examination.

Glibenclamide is an oral antidiabetic arrangement with a productive hypoglycemic activity. Diaonil (Glibenclamide) (S.K.Prasad et.al, 2009) fabricated by Aventis Pharma Ltd. Goa, India, was gathered from market and safeguarded at room temperature.

Induction of Experimental Diabetes

The grown-up pale skinned person wistar rodents (150-250gm) were medium-term fasted and decide the fasting blood glucose level. The arrangement blood glucose level of creatures were chosen and with the exception of gathering I creatures used to instigate diabetes by single i.p infusion of 120 mg/kg of Alloxan monohydrate was broken up in ordinary saline (pH-4.5).

Creatures were bolstered with 5% glucose arrangement so as to avert hypoglycemic stun for 18 hrs (Prince PSM et al., 1989). Hyperglycemia is to be affirmed the raised blood glucose levels, decided at 72 hrs and afterward on day 0 after infusion. The limit benefit of fasting blood glucose level >200mg/dl was taken as diabetic creature and rodents found with perpetual diabetes were utilized for the antidiabetic ponder.

Experimental Design

Exploratory rodents were partitioned into 5 gatherings of six creatures every all the gathering of creatures were prompted diabetic aside from control and treated for 21 days as pursues.

Group I: Normal control rodents nourished with vehicles as it were. (Typical saline with 1% CMC)

Group II: Diabetic controls rodents (Alloxan monohydrate 120mg/kg body weight of rodents, once i.p infusion).

Group III: Diabetic rodents treated with standard medication, Glibenclamide 3mg/kg per oral body weight.

Group IV: Diabetic rodents treated with ethanolic concentrate of *Rhinacanthus Nasutus* 200mg/kg, per oral, disintegrated in 1% carboxy methyl cellulose (CMC).

Group V: Diabetic rodents treated with ethanolic concentrate of *Rhinacanthus Nasutus* 400mg/kg, per oral, broke down in 1% carboxy methyl cellulose (CMC).

Sample collection

Fasting blood glucose (FBG) of all rodents was resolved

before the beginning of the examination. Blood test was gathered at week after week interims from tail vein cut till the finish of study. In the constant 21 days of medication treatment, a blood glucose level of all creatures was resolved at the 0, 7, 14, multi day by utilizing one touch glucometer (SD Check) technique.

On day 21, medium-term fasted creatures were under gentle ether anesthesia, the blood was gathered by direct heart cut. Blood was gathered in cylinders containing EDTA as anticoagulant for estimation of fasting plasma glucose and HbA1c.

Plasma was isolated by centrifugation at 3000 rpm for 10 minutes, the reasonable supernatant dull fluid was utilized for the investigation of different biochemical parameters. The pancreas tissues were extracted and flushed in super cold saline and kept in formalin answer for further histopathological examines.

Evaluation of Parameters

Estimation of changes in body weight of the animals

Body weight of all rats was measured on starting day (0 day) of the test and 21 day of the test. Both starting and final body weights were noted and reported.

Estimation of blood glucose level

Glucose level in Blood was measured by glucose oxidase / peroxidase technique using a commercial kit from Med source Ozone Biomedicals Pvt Ltd followed by Trinder, p.(1969) Annals. Clin. Bio chem.

Reagents

1. Enzyme reagent
2. Buffer solution
3. Glucose standard (100 mg%)

Procedure

10 µl of blood was added to 1.0 ml of working enzyme reagent, mixed well and incubated at 37°C for 15 min. The color settled was read at 505 nm against blank holding distilled water in its place of the sample. A standard was also processed similarly. The level of glucose is stated as mg/dl.

Statistical Analysis

Every one of the estimations of body weight, fasting blood glucose level, and biochemical parameter estimations were communicated as mean ± standard mistake of mean (S.E.M) and was broke down for centrality by ANOVA and gatherings were analyzed by Tukey-Kramer multiple correlation test, utilizing InStat v.2.02 programming (GraphPad Software Inc.). Contrasts between gatherings (p Value) were viewed as noteworthy at P<0.05 level. All information were graphically spoken to by utilizing Prism Software V 2.02.

RESULTS AND DISCUSSION

As described earlier in the methodology, the current study entitled "Pharmacological screening of anti-

diabetic activity of sapindus emarginatus” was carried out. Based on literature review the leaves of Sapindus Emarginatus selected and project work was carried out in Sapindus Emarginatus to the family Sapindaceae collected and authenticated. The result of the present study show that the ethanol extract exerts anti-diabetic Sapindus Emarginatus effect against alloxan induced diabetes. The obtained blood sugar levels of diabetic rats are as follows:

Diabetic screening

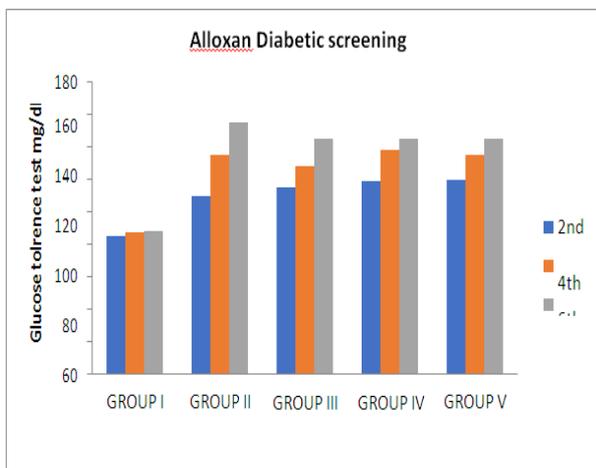


Table 6: Data of fasting glucose in Alloxan Treated Rats.

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Day	Blood Glucose Levels (mg/dl)				
	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
2 nd	85	110	115	119	120
4 th	87	135	128	138	135
6 th	88	155	145	145	145

Table 7: Data of Random blood glucose levels in Alloxan Treated Rats.

Day	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
2nd	180	230	235	222	220
4th	190	255	250	255	240
6th	188	265	270	265	268

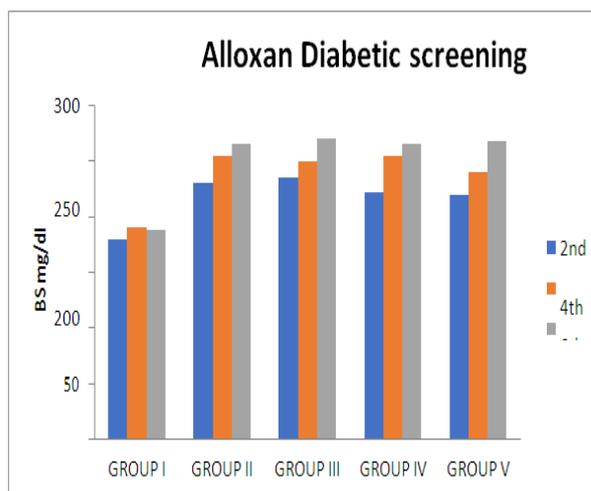


Fig. 4: Comparison of Fasting Blood Glucose Level In Alloxan Treated Rats

Random Blood glucose levels.

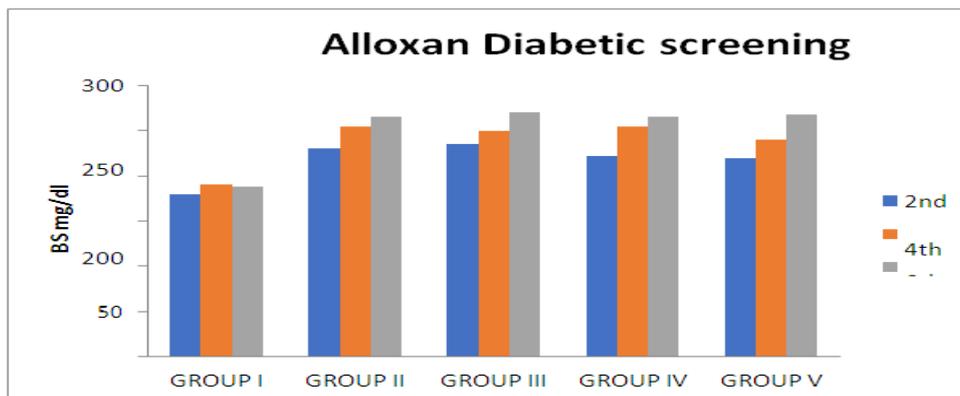


Fig. 5: Comparison of Random Blood Glucose Level In Alloxan Treated Rats.

Pharmacological Studies Acute Oral Toxicity Studies

The acute oral toxicity of the ethanolic extract of *Sapindus Emarginatus* was done as per OECD 423-guidelines (Acute toxic class method). Acute toxicity studies exposed that LD50>2000mg/kg for the extract. Hence, the biological dose was fixed at EESE 200mg and 400 mg of body weight for the extract.

Effect on Glucose Tolerance

In OGTT, the doses of EESE 200 mg/kg and 400 mg/kg improved the tolerance for glucose suggesting improved peripheral utilization of glucose. The reduction in blood glucose level was dose dependent.

The results were given in Table No.: 8 and Figure No.: 6

Table 8: Effect of Ethanolic extract of *Sapindus Emarginatus* and Glibenclamide on Glucose tolerance of diabetic rats.

Groups	Treatment	Change in blood glucose levels(mg/dL)			
		Fasting	After 30 Minutes	After 60 minutes	After 90 Minutes
I.	Glucose 2mg/kg	83.35±3.60	124±2.90	126.42±2.10	115.6±2.66
II.	Glibenclamide 3mg/kg	77.55±3.13	80.92±3.8 a	71.44±2.27 a	65.4±3.60 a
III.	EESE 200mg/kg	69.43±1.22	122.57±5.82 a	108.23±11.00 a	89.97±5.99 a
IV.	EESE 400mg/kg	88±3.28	110±2.61 c	102.25±7.91 b	89.45±3.25 a

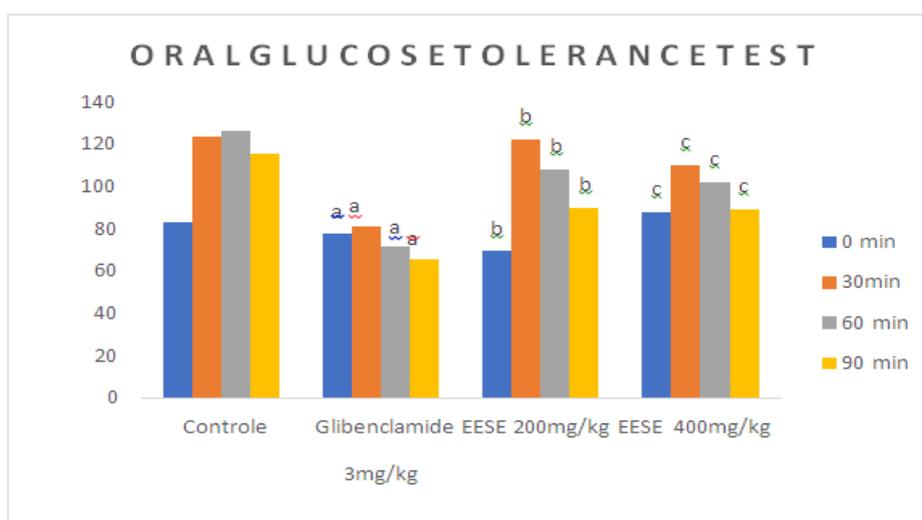


Fig. 6: Effect of ethanolic extract of *Sapindus Emarginatus* and Glibenclamide on glucose tolerance of diabetic rats.

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.05; b=** = p>0.05; c=* =p>0.05. Extract treated group III, IV were compared with group I (Normal) and Group II (Standard).

observed in diabetic control rats when compared to control rats. Administration of EEMK and *Glibenclamide* to diabetic rats significantly decreased the elevated level of blood glucose, near to control level. Shown Table No: 9, Figure No: 7.

Changes in blood glucose

A significant increase in the level of blood glucose, was

Table 9: Effect of ethanolic extract of *Sapindus Emarginatus* and Glibenclamide on blood glucose level.

Group	Treatment	Blood glucose level (mg/dL)			
		Day 0	Day 7	Day 14	Day 21
I	Normal control rats (vehicles only)	70.65 ± 1.42	80±2.34	78.83 ± 2.36	72.33 ±1.82
II	Diabetic control rats	380.6±13.57a	336.84±7.18 a	354.84±10.81a	369.32±12.91a
III	Diabetic group +Glibenclamide 3mg/kg	313.6±9.09 a	281.34±9.56 a	233.65±5.42a	147.67±8.05a
VI	Diabetic group +EESE (200mg/kg)	334.66±8.90b	285±13.26 a	174.82±8.91a	161±10.81 a
V	Diabetic group +EESE (400mg/kg)	321.84±12.16b	286±5.08 b	157.82±7.30a	160.5±7.74 a

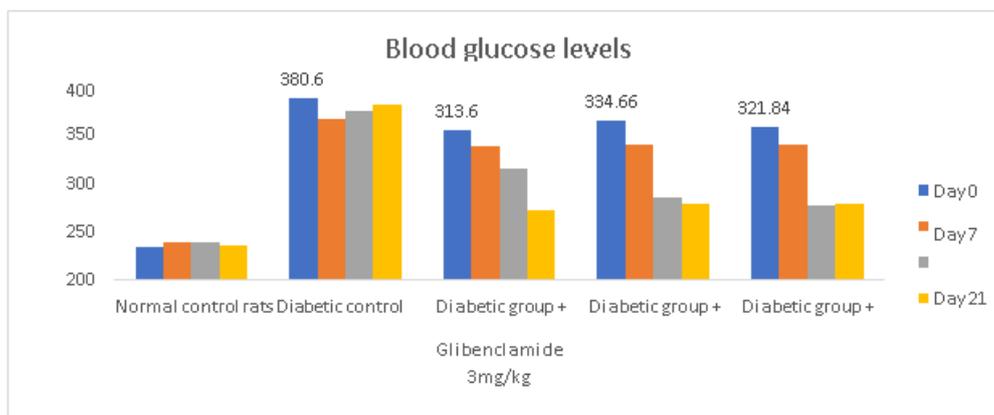


Figure 7: Effect of ethanolic extract of *Sapindus Emarginatus* and Glibenclamide on blood glucose level.

Values are given as mean \pm S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p > 0.001$; b=** = $p > 0.05$; c=* = $p > 0.05$. Extract treated group III, IV were compared with group I (Normal) and Group II (Standard).

SUMMARY AND CONCLUSION

The leaves of *Sapindus Emarginatus* belonging to family Sapindaceae has been examined to gain an insight of its pharmacological behaviors.

The pharmacological and acute toxicity studies of ethanolic extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed up to 2000mg/kg of body weight. The Biological dose of extract *Sapindus Emarginatus* dose was selected 200mg/kg and 400mg/kg in this dose possessed significant antidiabetic activity.

In conclusion, in the present study on the ethanolic extract of *Sapindus Emarginatus* leaves having antidiabetic activity more over nearest activity of *Glibenclamide*. This study shows that flavanoids present in this extract may be possibly responsible for the antidiabetic activities.

Further pharmacological and biochemical investigation are to be done to find out the active constituent responsible for the antidiabetic activity. However, the future study may also include cataloging, standardizing, for quality control and above all developing new drugs/ pharmaceuticals keeping the disease and cost factor in view.

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