Journal of Cardiovascular Disease Research

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

Phyllanthus niruri and *acidus Ethanolic* Extracts: Potential Hypolipidemic and Hypoglycemic Effects

Dr. Sagar N Firke[1], Pranjali Bajrang Chole[2], Dr. Umabati Sahu[3], Suraj Mandal[4], Dr Mohit Kotnala[5], Dr. Mukesh Kumar Meena[6], Jatoth Manjula[7], Dr. Nihar Ranjan Kar[8], Dr. P. Poli Reddy[9]*

[1]Assistant Professor, SSBES, Nanded Pharmacy College. Syam Nagar Nanded 431605, Maharashtra
[2]Research Scholar, CHRIST (Deemed to be University), Bangalore, Karnataka.
[3]Assistant professor of Botany, Fakir Mohan Autonomous College,Balasore , Odisha,PIN - 756001
[4]Science Teacher, Bloom Charter Education, Ain Al Fayedah School-6021 Abu Dhabi United Arab Emirates.
[5]Assistant Professor, Department of Pharmacy, IIMT College of Medical Sciences, IIMT University, O-Pocket, Ganganagar, Meerut, 250001, U.P., India
[6]Assistant Professor, Department of Pharmaceutical Sciences, Mohanlal Sukhadia University, Udaipur, Rajasthan-313001
[7]Assistant Professor, Gyanajyothi college of pharmacy uppal Hyderabad 500092
[8]Assistant Professor, Centurion University of Technology and Management, Gopalpur, balasore, Odisha, India, Pin-756044
[9]Associate Professor, Nalanda College of Pharmacy, Nalgonda, Telangana -508001
Corresponding Author Details:Dr. P. Poli Reddy,
polireddy0004@gmail.com

Abstract:
The major goals of this research were to evaluate ethanolic extracts from *Phyllanthus niruri* and *Phyllanthus acidus* in a number of animal models for their early antihyperglycemic and anti-hyperlipidemic effects. The present study looked at the effects of ethanolic extracts from Phyllanthus niruri and Phyllanthus acidus on glucose overload in hyperglycemic rats as well as in normal rats that had fasted overnight. Furthermore, in order to assess the potential antihyperlipidemic characteristics of the extract, the researchers employed rats that had been given Triton injections to induce hyperlipidemia. For each unique method, the experimental procedures used two different dosage levels, 200 and 400 mg/kg. With a sample size of 6, the results of the research were reported to have a mean standard error. With a significance threshold set at 5% (P0.05), the data were statistically analysed using a one-way analysis of variance (ANOVA) and the Bonferroni Multiple Comparison Test. When rats were given a dosage of 400 mg/kg of the ethanol extract made from Phyllanthus niruri and Phyllanthus acidus, their blood glucose levels were dramatically lowered (P 0.05). The research also found that rats with hyperlipidemia showed a reduction in their blood lipid profile, including total cholesterol, triglycerides, low density lipoprotein (LDL), and very low density lipoprotein (VLDL), in a way that was reliant on the dose given (P 0.05). High density lipoprotein (HDL) levels rose in tandem with this decline. The ethanolic extract made from these plants has been shown in this investigation to have hypolipidemic and hypoglycemic effects. These findings support the current scientific agreement about the effectiveness of this extract. By incorporating this substance into conventional therapies, it may be possible to control issues caused by

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

hyperglycemia and hyperlipidemia more effectively. Alternatively, it may serve as an adjuvant therapy in addition to established, generally accepted practises.

Keywords: *Antihyperglycemic, anti-hyperlipidemic, lipid profile, Phyllanthus niruri andPhyllanthus acidus*

Introduction:

Diabetes mellitus (DM) encompasses a group of intricate metabolic disorders that impact the metabolism of carbohydrates, lipids, and proteins. These disorders are distinguished by the presence of hyperglycemia, which arises from a shortage in the synthesis and/or effectiveness of insulin, or both [1].According to the figures provided by the International Diabetes Federation, the current prevalence of diabetes among adults aged 20 to 79 is around 537 million. It is projected that the prevalence of this condition would increase to 643 million individuals between the years 2030 and 2045. Impaired glucose tolerance (IGT) is a condition that impacts a population of about 1.541 million individuals, hence increasing their susceptibility to the development of type 2 diabetes [2].An further concern is to the growing prevalence rates of obesity and overweight, which pose substantial public health challenges. The worldwide prevalence of overweight and obesity is estimated to impact around 650 million and 1.9 billion individuals, respectively [3]. Furthermore, it is worth noting that a significant proportion, exceeding 60%, of the global population is concentrated on the Asian continent, with the majority of individuals residing in the countries of China and India [4]. The presence of obesity is associated with an increased susceptibility to several health diseases, including diabetes mellitus (DM), cancer, heart disease, as well as musculoskeletal and neurological disorders [5]. Consequently, these factors have a detrimental effect on the well-being of individuals residing in the area, while also directly impeding the overall economic performance and productivity of the country.

Several pharmaceutical drugs often prescribed for the treatment of obesity and diabetes are associated with adverse effects such as lactic acidosis, hyperglycemia, diarrhoea, and flatulence, which impose a costly burden on individuals [6].In order to mitigate the adverse effects and financial burden, supplementary therapeutic strategies are being employed alongside the primary pharmacological treatment for type II diabetes mellitus. This treatment regimen encompasses a range of medications including insulin secretagogues, biguanides, insulin sensitizers, α-glucosidase inhibitors, incretin mimetics, amylin antagonists, and sodium-glucose co-transporter-2 (SGLT2) inhibitors.The existing limitations of present dosage forms include the need for frequent administration, resulting in heightened adverse reactions and reduced patient adherence. These limits are attributed to the diverse bioavailability and limited duration of action shown by these forms.

The management of obesity may be facilitated via the implementation of increased physical activity and the adoption of a reasonable dietary approach that emphasises the intake of foods that are rich in fat and sugar [7]. Throughout history, Indian traditional medicinal herbs and their derived plant products have been used for the purpose of treating various ailments, such as diabetes and hyperlipidemia. These substances are often used in the field of supplementary

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

medicine. Purified and unprocessed plant extracts have a high concentration of active phytochemicals, which regulate many signalling pathways to manifest their anti-inflammatory and antioxidant properties [8, 9]. The plant-based products mentioned in the study are readily accessible, generally embraced, and cheaply priced [10]. Furthermore, it should be noted that these interventions have been shown to be both safe and efficacious.

The antiviral actions of Phyllanthus niruri have been attributed to its many compounds, such as flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, and saponins. These elements have also been associated with the plant's antibacterial, hepatoprotective, anticancer, and hypocalcemic capabilities [11].The flavonoids rutin and quercetin, which are obtained from P. niruri, are well acknowledged for their strong antioxidant and chelating properties [13].

Phyllanthus acidus (Phyllanthaceae), also known as "star gooseberry," is a little tree that is cultivated as a fruit-bearing plant in many Asian countries. According to a source [14], the leaves of this particular plant have been purportedly used for the treatment of conditions such as blood vomiting, piles, smallpox, and fever.

Nevertheless, it has been suggested that the aforementioned plants possess antidiabetic effects, despite the absence of empirical evidence to substantiate this claim. Hence, an investigation was conducted to examine the initial anti-hyperglycemic and anti-hyperlipidemic effects of ethanol extracts derived from Phyllanthus acidus and Phyllanthus niruri in several animal models.

Methods

Preparation of extracts

A sequential extraction procedure was used to extract dried and powdered plant components weighing 400 grammes, using a soxhlet equipment. The selection of solvents was based on their varying degrees of polarity, such as Petroleum Ether (60-80°C), Chloroform, and Ethanol. The extraction process was conducted over a period of 72 hours for each solvent. The extracts were subjected to drying processes using a rotating vacuum evaporator and freeze dryer. The percentage yields were obtained and afterwards kept in a desiccator for future use.

Phytochemical screening

Various extracts derived from the aforementioned extraction process were subjected to analysis in order to determine the presence of different phytoconstituents, including alkaloids, glycosides, flavones, tannins, terpenes, sterols, saponins, fats, and sugars. This analysis was conducted using the method of preliminary phytochemical study, specifically employing colour reactions, as detailed in previous literature references [15, 16, 17].

Acute toxicity studies

The OECD's (Organisation for Economic Co-operation and Development) guidelines were followed while assessing the acute oral toxicity of extracts. Young female albino mice were

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

given escalating dosages of dried extracts from Phyllanthus niruri and Phyllanthus acidus as part of the investigation. 100 mg/kg to 2000 mg/kg was the range of doses. The investigated animals were thoroughly watched by the researchers for any signs of toxicity [18].

The albino mice were divided into a number of cohorts, each with six distinct individuals. The dosage of distilled water administered orally to the control group was 5 ml/kg. The remaining groups received oral administration of Phyllanthus niruri and Phyllanthus acidus ethanolic extracts at different dose levels, including 100, 500, 1000, 1500, and 2000mg/kg.

The animals were kept under constant observation for 4 hours after the dose was administered, with sporadic monitoring lasting up to 24 hours. Any incidences of mortality were noted at the end of the 72-hour period [19]. Additional observations recorded by the researchers include behavioural changes, somatomotor activity, tremors, convulsions, tonic extension, stub tails, muscle spasms, loss of the righting reflex, ataxia, sedation, hypnosis, lacrimation, diarrhoea, salivation, writhing, and changes in the skin, fur, eyes, and mucous membranes [19]. One-tenth of the upper limit dosage, as well as its half and double dosages, were employed to assess therapeutic activity.

Oral Glucose Tolerance Test

According to the procedure outlined by Jarald et al., 2008 [20], the OGTT was carried out in overnight-fasted normal rats after acclimatisation for seven days in the departmental laboratory.The rats were randomly assigned to seven groups of six rats each, and various medications were given to them in accordance with the schedule shown in Table 1.

Table 1: Schedule of drug administration in different groups of OGTT

Groups	Treatment groups	Treatments and Dose
Group-I	Normal control	Distilled Water (5 ml/kg)
Group-II	Glucose loaded Control	Distilled Water (5 ml/kg) + Glucose (2 g/kg)
Group-III	Standard	Glibenclamide (5 mg/kg) + Glucose (2 g/kg)
Group-IV	EEPN-200	EEPN (200 mg/kg) + Glucose (2 g/kg)
Group-V	EEPN-400	EEPN (400 mg/kg) + Glucose (2 g/kg)
Group-VI	EEPA-200	EEPA (200 mg/kg) + Glucose (2 g/kg)
Group- VII	EEPA-400	EEPA (400 mg/kg) + Glucose (2 g)/kg

EEPN=Ethanolic extracts of *Phyllanthus niruri* and EEPA=Ethanolic extracts of *Phyllanthus acidus*

The present study aimed to explore the antihyperglycemic activity in rats with hyperglycemia induced by glucose excess. The rats were administered several drugs in accordance with a predetermined schedule after a 12-hour period of fasting, during which they were given unrestricted access to water. The blood glucose concentration at the start of the experiment was determined in rats that had been fasted overnight. After a 30-minute period of drug treatment,

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

all groups of rats were given oral glucose feedings at a dosage of 2 gm/kg (p.o.). Blood glucose levels were monitored after a period of glucose loading lasting 30, 60, 90, and 120 minutes. Blood samples were collected using rat tail tips, and the glucose concentration was afterwards measured by using a Glucometer together with Glucometer strips.

Hypoglycemic activity

The experiment on hypoglycemic activity was conducted on normal rats that had been fasted overnight, following the procedure outlined by Jarald et al. in 2008 [20]. Following a period of acclimatisation lasting seven days inside the departmental laboratory, the rats were then allocated into six groups, each consisting of six rats. The administration of medicines was carried out in accordance with the predetermined schedule outlined in Table 2.

Table 2: Schedule of drug administration in different groups of hypoglycemic activity study

Groups	Treatment groups	Treatments and Dose
Group-I	Normal Control	Distilled Water (5 ml/kg)
Group-II	Standard	Glibenclamide (5 mg/kg)
Group-III	EEPN-200	EEPN (200 mg/kg)
Group-IV	EEPN-400	EEPN (400 mg/kg)
Group-V	EEPA-200	EEPA (200 mg/kg)
Group- VI	EEPA-400	EEPA (400 mg/kg)

The study focused on examining the hypoglycemia activity in rats with normal physiological conditions. The rats had a 12-hour period of fasting, during which they had unrestricted access to water. Following this, the rats were given various medicines according to the predetermined timetable, with each drug being supplied to the appropriate group. The minimum blood sugar level of zero was established by measuring the blood sugar levels of animals after an overnight fast, prior to the administration of the medication orally. The blood glucose concentration was assessed at various time intervals (30, 60, 90, and 120 minutes) subsequent to the oral administration of the medication. The blood samples were obtained from the rats' tail tips and the glucose content was evaluated using a glucometer.

Triton-induced hyperlipidemic model

Multiple studies have shown that the systemic injection of Triton WR 1339, an ionic surfactant, to fasting rats results in an increase in plasma lipid levels. The use of Triton WR-1339 has been extensively employed in inducing acute hyperlipidemia in several animal models by impeding the clearance of triglyceride-rich lipoproteins [21]. The paradigm described in this study is extensively used in other animal species [22]. Specifically, in the case of rats, it has been utilised for the evaluation of natural or artificial hypolipidemic medicines [23].

Following a period of 7 days for acclimation, an investigation was conducted to examine the hypolipidemic activity in rats with hyperlipidemia produced by triton WR 1339. The rats were allocated randomly into six groups, each consisting of six rats. The medications were delivered to the rats according to the specified schedule outlined in Table 3.

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

Table 3: Schedule of drug administration in different groups of triton induced hyperlipidemic study

Groups	Treatment Groups	Treatments and Dose
Group-I	Normal control	Distilled water- 10 ml/kg
Group-II	Hyperlipidemic control	Distilled water- 10 ml/kg + Triton WR1339 (50mg/kg)
Group-III	Standard	Simvastatin-15mg/kg + Triton WR1339 (50mg/kg)
Group-IV	EEPN-200	EEPN - 250 mg/kg + Triton WR1339 (50 mg/kg)
Group-V	EEPN-400	EEPN - 450 mg/kg + Triton WR1339 (50 mg/kg)
Group-VI	EEPA-200	EEPA - 250 mg/kg + Triton WR1339 (50 mg/kg)
Group-VII	EEPA-400	EEPA - 450 mg/kg + Triton WR1339 (50 mg/kg)

The administration of drugs and plant extracts was conducted orally to the different groups, both immediately and 20 hours after the intraperitoneal injection of Triton WR 1339. The rats were subjected to a fasting state, while being provided unrestricted access to water for the duration of the experiment. After a duration of 4 hours after the administration of the second dosage, blood samples were obtained from all animals and then used for the examination of lipid profiles, namely serum cholesterol, triglycerides, and HDL cholesterol. This analysis was conducted utilising an automated analyzer and commercially accessible kits. The calculation of LDL cholesterol and VLDL was performed using Friedewald's algorithm [24].

Statistical analysis

The values are expressed as mean ± SEM. The results were analyzed for statistical significance using one-way ANOVA (and nonparametric), followed by Bonferroni's Multiple Comparison Test (Graph pad prism 5.04 version). $P \leq 0.05$ was considered statistically significant.

Results And Discussion

Preparation of extracts

The extraction was carried out by dried pulverized plant materials of 400 gm of *Phyllanthus niruri and Phyllanthus acidus* the results are given in Table 4.

Table 4: Percentage yield (w/w) of different extracts

Solvents	Percentage of yield (w/w) of Different Extracts	
	Phyllanthus niruri	***Phyllanthus acidus***
Pet. Ether	3.4%	4.1%
Chloroform	6.7%	7.2%
Ethanol	13.7%	11.3%

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

The percentage yields of petroleum ether, chloroform, and ethanol extract of *Phyllanthus niruri* were found to be 3.4%, 6.7%, and 13.7% w/w respectively; and of *Phyllanthus acidus*were found to be 4.1%, 7.2%, and 11.3%w/wrespectively.

Phytochemical screening

A summary of the results of preliminary phytochemical screening of ethanolic extracts of *Phyllanthus niruri andPhyllanthus acidus* are furnished in the Table 5.

Table 5: Preliminary phytochemical investigation of different extracts

Group of phytoconstituents	**Ethanolic extract of *Phyllanthus niruri***	**Ethanolic extract of *Phyllanthus acidus***
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	+	+
Cardiac glycosides	+	+
Anthraquinone glycosides	+	-
Saponin glycosides	+	+
Gums and mucilage	-	-
Proteins and Amino acids	+	+
Tannins and phenolic compounds	+	+
Triterpenoids	+	+
Flavonoids	+	+
Coumarins	+	-
Steroids	-	-
Fats and oils	-	-

(+) Sign indicates presence, (—) Sign indicates absence

The phytochemical studies of EEPN and EEPA contains phenolic compounds such as flavons, flavonoids, tannins etc. Several papers have recently reported the hypoglycemic and hypolipidemic effects of phenolic compounds such as flavonoids, flavons, tannins etc. Hence, the EEPN and EEPA were used for further studies.

Acute Toxicity Study

A summary of acute toxicity of ethanolic extracts of *Phyllanthus niruri* and *Phyllanthus acidus* are furnished in the Table 6.

Table 6: Acute toxicity studies of different extracts of *Phyllanthus niruri* and *Phyllanthus acidus*

Treatment	**Dose (mg/kg)**	**No. of Mice**	**No. of Deat**	**signs of toxicity**	**LD50**

			h		
Control (Distilled water)	10 ml/ kg	6	0	-	-
Ethanolic extracts of *Phyllanthus niruri* (EEPN)	100	6	0	-	> 2000 mg/kg
	500	6	0	-	
	1000	6	0	-	
	1500	6	0	-	
	2000	6	0	-	
Ethanol extract of *Phyllanthus acidus* (EEPS)	100	6	0	-	> 2000 mg/kg
	500	6	0	-	
	1000	6	0	-	
	1500	6	0	-	
	2000	6	0	-	

In each instance, no fatalities were recorded. Furthermore, it was observed that many physiological indicators, such as alterations in skin, fur, eyes, mucous membranes, respiratory and circulatory systems, as well as autonomic and central nervous systems, somato-motor activity, and behavioural patterns, exhibited normal characteristics. Furthermore, careful consideration was paid to the examination of tremors and convulsions, which were shown to be absent in all experimental groups. The documented findings may be seen in Table 6.

The results of the acute toxicity investigation indicated that the ethanol extracts derived from Phyllanthus niruri and Phyllanthus acidus did not exhibit any indications of toxicity or death, even when administered at a dosage level of 2000 mg/kg body weight.According to the European Economic Community (EEC) classification system for acute oral toxicity, substances with LD50 doses of 2000 mg/kg and higher are classed as unclassified and exhibit low toxicity, as outlined in the EC Directive 83/467/EEC of 1983.

The overall findings indicate that the LD50 value exceeds 2000 mg/kg. Therefore, the therapeutic dosage was determined to be 1/10th of the maximum acceptable dose, which is equivalent to 200 mg/kg of body weight. Consequently, further pharmacological tests were conducted with doses of 1/5th (400 mg/kg) and 1/10th (200 mg/kg) of the initial dose of 2000 mg/kg [18-40].

The hypoglycemic and hypolipidemic effects of the ethanol extracts from Phyllanthus niruri and Phyllanthus acidus were assessed separately by experimentation on several animal models.

The effect of ethanolic extracts in glucose loaded hyperglycemic animals:

The glucose concentration was estimated at 0, 30, 60, 90 and 120 minutes after the glucose loading and results are recorded in Table 7, 8 and Figure 1.

Table7: Effect of different extracts on blood glucose concentration in glucose loaded rats

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

Groups	Mean blood glucose concentration (mg/dl) at different time				
	0 min	30 min	60 min	90 min	120 min
Normal control	86.83 ± 2.72	86.17 ± 2.51	88.67 ± 2.14	87.83 ± 3.74	84.33 ± 2.95
Glucose loaded Control	90.33 ± 3.63	148.17 ± 3.42[a]	161.83 ± 3.38 a	141.17 ± 3.66 [a]	126.17 ± 3.42 [a]
Standard	84.50 ± 3.77	112.83 ± 4.42 ***	93.17 ± 2.98***	78.83 ± 3.57***	68.83 ± 2.80***
EEPN-200	87.17 ± 3.39	#127.6 ± 73.07*	134.67 ± 4.14***	112.67 ± 3.07***	#101.17 ± 3.54***
EEPN-400	89.33 ± 1.78	#118.50 ± 3.21***	#107.83 ± 3.28***	#89.67 ± 3.20***	#80.50 ± 3.12***
EEPA-200	90.17 ± 4.55	134.33 ± 3.58	146.67 ± 2.75	122.83 ± 3.53*	109.83 ±2.79*
EEPA-400	86.83 ± 5.57	#124.17 ± 4.17**	#118.17 ± 3.94***	#95.67 ± 4.18***	#88.67 ± 3.23***

The results were expressed asMean ± SEM, n=6.

[a]P≤ 0.001, [b]P≤ 0.01 and [c]P≤ 0.05; compared Normal control vs Glucose loaded control.

****P≤ 0.001, **P≤ 0.01 and *P≤ 0.05; compared Standard and Test groups vs Glucose loaded control.*

'#'- Indicates there is no significant difference between standard and test drug at P≤ 0.05 significant level.

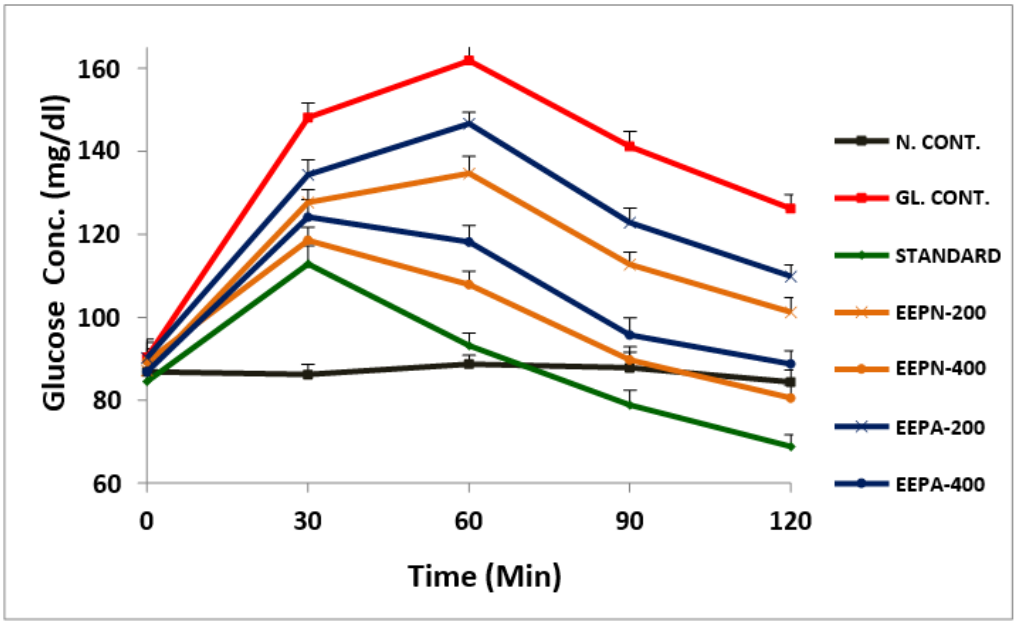

Figure 1: Effects of ethanol extracts on blood glucose concentration

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

Table 8: Percentage change in blood glucose concentration of different groups

Groups	Percentage changes in blood glucose (mg/dl) at different time				
	0 min	30 min	60 min	90 min	120 min
Standard	6.46	23.85	42.43	44.16	45.44
EEPN-200	3.51	13.84	16.79	20.19	19.82
EEPN-400	1.11	20.02	33.37	36.48	36.20
EEPA-200	0.18	9.34	9.37	12.99	12.95
EEPA-400	3.87	16.20	26.98	32.23	29.72

In the context of oral glucose tolerance testing (OGTT), the antihyperglycemic effect was shown in hyperglycemic rats. Following the administration of glucose, a notable increase in blood glucose levels was observed in the control group. However, after a duration of two hours, a drop in glucose levels was observed. The experimental ethanolic extract of Plant Name (EEPN) and its active compound, Ethanol Extract Plant Agent (EEPA), demonstrated a significant reduction in blood glucose levels at doses of 200 and 400 mg/kg after the administration of glucose, as compared to the control group of animals. No statistically significant difference was seen between the administration of 400 mg/kg of both extracts and the treatment of rats with glibenclamide.

The hypoglycemic effect of ethanolic extracts in fasted normal rats:

The glucose concentration was estimated at 0, 30, 60, 90 and 120 minof the drug administration and results wererecorded in Table 9, 10 and Figure 2.

Table 9: Effect of different extracts on blood glucose concentration in overnight fasted normal rats

Groups	Mean blood glucose (mg/dl) at different time				
	0 min	30 min	60 min	90 min	120 min
Normal Control	84.50 ± 2.88	86.33 ± 3.24	83.67 ± 2.72	82.67 ± 2.14	85.83 ± 3.04
Standard	83.17 ± 3.27	64.83 ± 3.75***	57.67 ± 2.74***	52.83 ± 2.27***	48.17 ± 2.21***
EEPN-200	84.67 ± 3.22	78.33 ±2.67#	74.83 ± 3.86	77.17 ± 3.09##	81.17 ± 3.22###
EEPN-400	82.67 ± 3.17	77.33 ± 1.54	76.83 ± 2.69	73.67 ± 3.86*,###	72.33 ± 3.82, ###
EEPA-200	85.17 ± 2.51	81.83 ± 2.64##	79.67 ± 2.26	74.17 ± 2.89*,###	77.83 ± 2.71,###

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

EEPA-400	83.17 ± 4.45	75.17 ± 2.47	69.17 ± 2.75*	64.83 ± 2.82**	61.67 ± 2.72***

The results were expressed as mean ± SEM, n=6.

****P≤ 0.001, **P≤ 0.01 and *P≤ 0.05; compared Standard and Test groups vs Normal control group.*

'#' P≤0.05, ##P≤0.01, P≤0.001 standardvs test drug

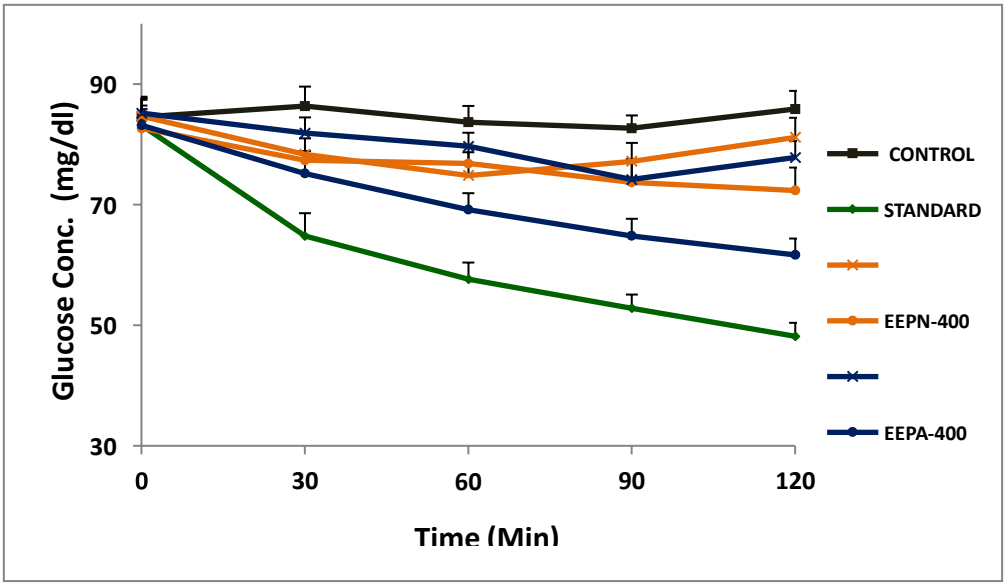

Figure 2: Effects of ethanol extracts on blood glucose concentration

Table 10: Percentage change in blood glucose concentration of different groups

Groups	Percentage change in blood glucose conc. at different time				
	0 min	30 min	60 min	90 min	120 min
Standard	1.56	29.05	45.63	52.39	58.55
EEPN-200	-0.20	9.27	10.56	6.65	5.44
EEPN-400	2.17	10.42	8.17	10.89	15.73
EEPA-200	-0.79	5.21	4.78	10.28	9.32
EEPA-400	1.58	12.93	17.33	21.57	28.16

The hypoglycaemic effect in fasted normal rats were evaluated. After 30 min. of drug administration to the end of 2 hours the blood glucose levels of the standard animals were declined. The EEPA extract at 400 mg/kg were shown hypoglycemic activity at 400 mg/kg dose level.

The effect of ethanolic extracts in Triton-induced hyperlipidemic model:

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

The lipid profile was estimated after administration of triton WR1339 and results were recorded in Table 11, 12 and Figure 3.

Table 11: Effect of different extracts on lipid levels in triton induced hyperlipidemic rats

Groups	Lipid profiles (mg/dl)				
	Total Cholesterol	**Triglycerides**	**HDL**	**LDL**	**VLDL**
Normal control	60.83 ± 1.54	54.17 ± 1.64	31.67 ± 1.28	28.13 ± 2.16	12.03 ± 0.33
Hyperlipide miccontrol	187.17± 3.58[a]	112.33 ± 1.93[a]	16.83 ± 1.11[a]	147.47 ± 4.42[a]	21.87 ± 0.39[a]
Standard	116.83± 2.97***	78.67 ± 2.33***	28.83 ± 1.14***	74.07 ± 2.67***	14.93 ± 0.47***
EEPN-200	174.83± 2.57	107.17 ± 2.14	22.33 ± 1.23*	124.67 ± 2.14**	20.33 ± 0.43
EEPN-400	130.83 ± 2.23***	91.50 ± 3.06***	#24.83 ± 1.25**	#85.50 ± 2.85***	17.50 ± 0.61*
EEPA-200	150.17± 3.69**	95.33 ± 1.76**	#24.1 ± 7 1.42*	106.93 ± 3.84***	18.67 ± 0.35*
EEPA-400	# 127.83 ± 2.57***	#87.33 ± 2.67***	#27.33 ± 1.36***	#83.83 ± 3.52***	#16.4 ± 7 0.53*

The results were expressed as mean ± SEM, n=6.
[a]P≤ 0.001, [b]P≤ 0.01 and [c]P≤ 0.05; compared Normal control vs Hyperlipidemic control.

****P≤ 0.001, **P≤ 0.01 and *P≤ 0.05; compared Standard and Test groups vs Hyperlipidemic control.*
And '#'- Indicates there is no significant difference between standard and test drug at P≤ 0.05 significant level.

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

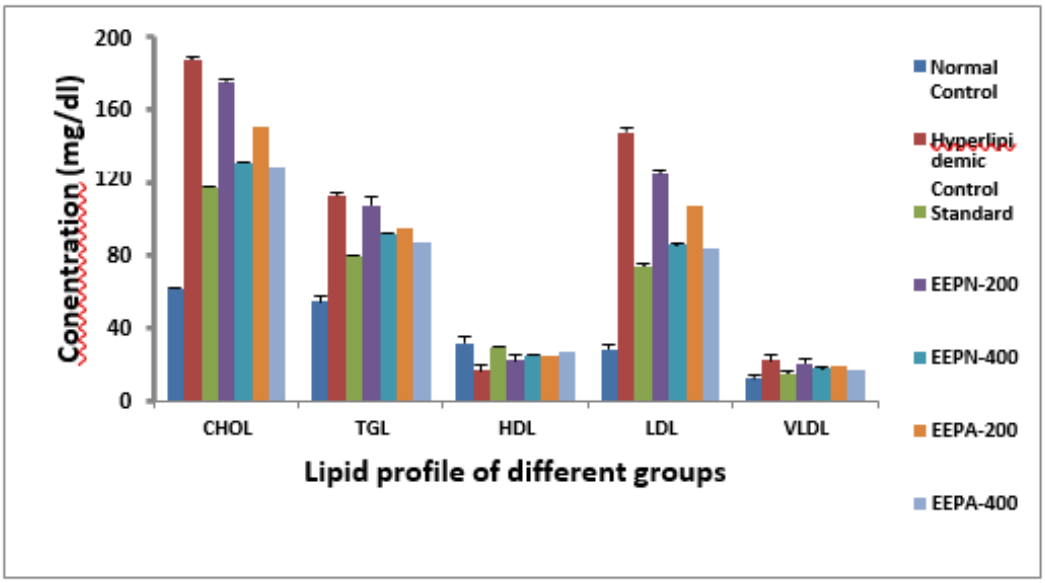

Figure 3: Lipid profiles i.e. Serum cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol andVLDL-cholesterol levels in differentdrug treatment groups

Table 12: Percentage change in lipid levels of different drug treatment groups vs hyperlipidemic control

Groups	Percentage changes in Lipid profiles				
	Total Cholesterol	Triglycerides	HDL	LDL	VLDL
Standard (simvastatin)	37.58	29.97	-71.29	49.77	31.71
EEPN-200	6.59	4.60	-32.67	15.46	7.01
EEPN-400	30.10	18.55	-47.52	42.02	19.97
EEPA-200	19.77	15.13	-43.56	27.49	14.63
EEPA-400	31.70	22.26	-62.38	43.15	24.70

As anticipated, the injection of Triton WR1339 resulted in an increase in blood lipid levels, which remained elevated throughout the duration of the trial in the hyperlipidemic control group. The antihyperlipidemic effects of EEPN and EEPA, administered at dosages of 200 and 400 mg/kg body weight, respectively, were shown to be similar to those of the reference standard Simvastatin. A notable increase in serum lipids was seen in Triton-induced hyperlipidemic control rats in comparison to the normal control group. The experimental group administered with EEPN and EEPA at dosage levels of 200 and 400 mg/kg respectively exhibited notable reductions in blood lipid levels in hyperlipidemic rats. These reductions were found to be statistically significant ($P \leq 0.05$) when compared to the hyperlipidemic control group.

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

Conclusion: The primary objective of this research was to investigate the potential hypoglycemic and hypolipidemic effects of EEPN and EEPA. The phytochemical analyses of EEPN and EEPA revealed the presence of phenolic compounds, including flavones, flavonoids, tannins, and other related substances. The results of the acute toxicity research indicate that both EEPN and EEPA exhibited no indicators of toxicity or death, even when administered at a dosage level of 2000 mg/kg body weight. The present study investigated the antihyperglycemic, hypoglycemic, and hypolipidemic effects in rats that were subjected to glucose loading, normal rats, and Triton WR1339-induced hyperlipidemic rats, respectively. The findings of the study indicate that EEPN and EEPA have promising potential in reducing blood glucose levels and also possess antihyperlipidemic properties. This study provides evidence to justify the incorporation of these botanical species into conventional formulations used for managing diabetes. Therefore, it is plausible to use it as a therapeutic agent or adjunct in current treatment for diabetes accompanied by hyperlipidemia, due to its potential hypoglycemic and hypolipidemic properties.

References

1. Vieira R, Souto SB, Sánchez-López E, Machado AL, Severino P, Jose S, Santini A, Fortuna A, García ML, Silva AM, Souto EB. Sugar-Lowering Drugs for Type 2 Diabetes Mellitus and Metabolic Syndrome-Review of Classical and New Compounds: Part-I. Pharmaceuticals (Basel). 2019 Oct 10;12(4):152. doi: 10.3390/ph12040152. PMID: 31658729; PMCID: PMC6958392.
2. IDF Diabetes Atlas . *Idf Diabetes Atlas.* International Diabetes Federation; Brussels, Belgium: 2021.
3. Nuertey BD, Alhassan AI, Nuertey AD, Mensah IA, Adongo V, Kabutey C, Addai J, Biritwum RB. Prevalence of obesity and overweight and its associated factors among registered pensioners in Ghana;a cross sectional studies. BMC Obes. 2017 Jul 4;4:26. doi: 10.1186/s40608-017-0162-4. PMID: 28690855; PMCID: PMC5496418.
4. Ramachandran A, Chamukuttan S, Shetty SA, Arun N, Susairaj P. Obesity in Asia--is it different from rest of the world. Diabetes Metab Res Rev. 2012 Dec;28 Suppl 2:47-51. doi: 10.1002/dmrr.2353. PMID: 23280866.
5. Chooi YC, Ding C, Magkos F. The epidemiology of obesity. Metabolism. 2019 Mar;92:6-10. doi: 10.1016/j.metabol.2018.09.005. Epub 2018 Sep 22. PMID: 30253139.
6. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. Int J Med Sci. 2014 Sep 6;11(11):1185-200. doi: 10.7150/ijms.10001. PMID: 25249787; PMCID: PMC4166864.
7. Fock KM, Khoo J. Diet and exercise in management of obesity and overweight. J Gastroenterol Hepatol. 2013 Dec;28 Suppl 4:59-63. doi: 10.1111/jgh.12407. PMID: 24251706.
8. Chandra S, Sah K, Bagewadi A, Keluskar V, Shetty A, Ammanagi R and Naik Z. Additive and synergistic effect of phytochemicals in prevention of oral cancer. Eur

J Gen Dent 2013; 1: 142-147.

9. Kalal BS, Fathima F, Pai VR, Sanjeev G, Krishna CM, Upadhya D. Inhibition of ERK1/2 or AKT Activity Equally Enhances Radiation Sensitization in B16F10 Cells. World J Oncol. 2018 Feb;9(1):21- 28. doi: 10.14740/wjon1088w. Epub 2018 Mar 8. PMID: 29581812; PMCID: PMC5862079.
10. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014 Jan 10;4:177. doi: 10.3389/fphar.2013.00177. PMID: 24454289; PMCID: PMC3887317.
11. Lee NY, Khoo WK, Adnan MA, Mahalingam TP, Fernandez AR, Jeevaratnam K. The pharmacological potential of Phyllanthus niruri. J Pharm Pharmacol. 2016 Aug;68(8):953-69. doi: 10.1111/jphp.12565. Epub 2016 Jun 10. PMID: 27283048.
12. Kaur N, Kaur B, Sirhindi G. Phytochemistry and Pharmacology of Phyllanthus niruri L.: A Review. Phytother Res. 2017 Jul;31(7):980-1004. doi: 10.1002/ptr.5825. Epub 2017 May 17. PMID: 28512988.
13. Rusmana, Djaja, et al. "Antioxidant Activity of Phyllanthus Niruri Extract, Rutin and Quercetin." *Indonesian Biomedical Journal*, vol. 9, no. 2, 2017, pp. 84-90, doi:10.18585/inabj.v9i2.281.
14. Tan SP, Tan EN, Lim QY, Nafiah MA. Phyllanthus acidus (L.) Skeels: A review of its traditional uses, phytochemistry, and pharmacological properties. J Ethnopharmacol. 2020 May 10;253:112610. doi: 10.1016/j.jep.2020.112610. Epub 2020 Jan 25. PMID: 31991202.
15. Kokate CK, Purohit AP, Gokhale SB. Text book of pharmacognosy. 33rd ed., Pune; Nirali Prakashan: 2001.
16. Khandelwal, KR. Practical Pharmacognosy. 14th ed., Pune; Nirali Prakashan: 2005. 146-55.
17. Evans WC. Trease and Evans Pharmacognosy. 15th ed., Edinburgh London, New York, Philadelphia, St Louis, Sydney, Toronto; W.B. Saunders: 2002.103-104, 227, 247-250, 336, 471, 519-520, 545-7.
18. Panigrahi, et al..Hypoglycemic And Hypolipidemic Activities Of Methanolic Extract Of *GlinusOppositifolius*. Int J Pharm 2012; 2(3): 491-497.
19. Ghosh MN. Fundamentals of Experimental Pharmacology. 3rd ed., Kolkata; Hliton& Company: 2005; 190-7.
20. Jarald EE, Joshi SB, Jain DC. Ind J Pharmacol, 2008; 40 (6); 256-60.
21. Schurr PE, Schultz JR, Parkinson TM. Lipids, 1972; 7: 69-74.
22. Li YY, Jiang CJ, Wan XC, Zhang ZZ, Li DX. Acta BiochimBiophys Sin, 2005; 37: 364- 70.
23. Harbowy ME, Balentine DA. Crit Rev Plant Sci, 1997; 16: 415-80.
24. Friedwald WT, Levy RT, Fredrickson DS. Clin Chem, 1972; 18: 499-502.
25. Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-s498.

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023

26. Pal N, Mandal S, Shiva K, Kumar B. Pharmacognostical, Phytochemical and Pharmacological Evaluation of Mallotus philippensis. Journal of Drug Delivery and Therapeutics. 2022 Sep 20;12(5):175-81.
27. Singh A, Mandal S. Ajwain (Trachyspermum ammi Linn): A review on Tremendous Herbal Plant with Various Pharmacological Activity. International Journal of Recent Advances in Multidisciplinary Topics. 2021 Jun 9;2(6):36-8.
28. Mandal S, Jaiswal V, Sagar MK, Kumar S. Formulation and evaluation of carica papaya nanoemulsion for treatment of dengue and thrombocytopenia. Plant Arch. 2021;21:1345-54.
29. Mandal S, Shiva K, Kumar KP, Goel S, Patel RK, Sharma S, Chaudhary R, Bhati A, Pal N, Dixit AK. Ocular drug delivery system (ODDS): Exploration the challenges and approaches to improve ODDS. Journal of Pharmaceutical and Biological Sciences. 2021 Jul 1;9(2):88-94.
30. Shiva K, Mandal S, Kumar S. Formulation and evaluation of topical antifungal gel of fluconazole using aloe vera gel. Int J Sci Res Develop. 2021;1:187-93.
31. Ali S, Farooqui NA, Ahmad S, Salman M, Mandal S. Catharanthus roseus (sadabahar): a brief study on medicinal plant having different pharmacological activities. Plant Archives. 2021;21(2):556-9.
32. Mandal S, Jaiswal DV, Shiva K. A review on marketed Carica papaya leaf extract (CPLE) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. International Journal of Pharmaceutical Research. 2020 Jul;12(3).
33. Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. Solanum Nigrum Linn: An Analysis Of The Medicinal Properties Of The Plant. Journal of Pharmaceutical Negative Results. 2023 Jan 1:1595-600.
34. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis Of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. Journal of Pharmaceutical Negative Results. 2022 Dec 31:9189-98.
35. Mandal S, Vishvakarma P, Mandal S. Future Aspects And Applications Of Nanoemulgel Formulation For Topical Lipophilic Drug Delivery. European Journal of Molecular & Clinical Medicine.;10(01):2023.
36. Chawla A, Mandal S, Vishvakarma P, Nile NP, Lokhande VN, Kakad VK, Chawla A. Ultra-Performance Liquid Chromatography (Uplc).
37. Mandal S, Raju D, Namdeo P, Patel A, Bhatt AK, Gupta JK, Haneef M, Vishvakarma P, Sharma UK. Development, characterization, and evaluation of rosa alba l extract-loaded phytosomes.
38. Mandal S, Goel S, Saxena M, Gupta P, Kumari J, Kumar P, Kumar M, Kumar R, Shiva K. Screening of catharanthus roseus stem extract for anti-ulcer potential in wistar rat.
39. Shiva K, Kaushik A, Irshad M, Sharma G, Mandal S. Evaluation and preparation: herbal gel containing thuja occidentalis and curcuma longa extracts.
40. Vishvakarma P, Kumari R, Vanmathi SM, Korni RD, Bhattacharya V, Jesudasan RE, Mandal S. Oral Delivery of Peptide and Protein Therapeutics: Challenges And Strategies. Journal of Experimental Zoology India. 2023 Jul 1;26(2).

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 08, 2023