ISSN 2582-6441 [Online]
RESEARCH JOURNAL OF PHARMACY AND LIFE SCIENCES
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An International Peer Reviewed Journal
RJPLS

**Research Article**

# PHARMACOLOGICAL EVALUATION OF LEAF AND ROOT EXTRACT OF *NEOLAMARCKIA CADAMBA* FOR HYPOGLYCEMIC ACTIVITY

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**ARTICLE INFO**

Date of submission: 18-07-2024
Date of Revision: 29-07-2024
Date of acceptance: 06-08-2024

**Key Words:** Hypoglycemic, *Neolamarckia Cadamba*, *Neolamarckia Cadamba*.

**ABSTRACT**

**Objective:** This study aimed to investigate the potential hypoglycemic effect of the ethanolic root and leaves extract of *Neolamarckia Cadamba* in rat model. **Methods:** Ethanolic root and leaves extract of *Neolamarckia Cadamba* at 200mg/kg and 400mg/kg doses were tested for antihyperglycemic activity in glucose overloaded hyperglycemic rats and hypoglycemic activity in overnight fasted normal rats.. The results of the study were expressed as mean ± SEM, n=6 and data was analyzed by using one way analysis of variance test (ANOVA) followed by Bonferroni's Multiple Comparison Test with 5% level of significance ($P<0.05$). **Results:** The ethanolic root and leaf extract of *Neolamarckia Cadamba* exhibited a significant reduction in fasting blood glucose levels ($P<0.05$) at 200 and 400 mg/ kg. **Conclusion:** The findings of this study demonstrate the potential hypoglycemic effect of the ethanolic root and leaf extract of *Neolamarckia Cadamba* in rats. These results suggested that *Neolamarckia Cadamba* may be a promising natural agent for the management of diabetes.



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## INTRODUCTION

Diabetes mellitus is a major endocrine disorder and growing health problem in most countries[1]. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades[2]. Diabetes mellitus is categorized as a metabolic disease characterized by common feature of chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The cause of type 2 diabetes is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response[3]. It was estimated that 2.8% of world population was diabetic in 2000 and this figure would climb to be as high as 4.4% of the world's population by 2030 (most of which will be type 2 diabetes mellitus)[4].

A number of plants are mentioned in ancient Indian literature for the treatment of hyperglycemic and hyperlipidemic conditions. One such drug is *Neolamarckia cadamba* of the family Rubiaceae, being used by some local tribal people, were selected for the present study. *Neolamarckia cadamba, with* English common names burflower-tree, laran, and Leichhardt pine, and called kadam or cadamba locally, is an evergreen, tropical tree native to South and Southeast Asia. The genus name honours French naturalist Jean-Baptiste Lamarck. It has scented orange flowers in dense globe-shaped clusters. The flowers are used in perfumes. The tree is grown as an ornamental plant and for timber and paper-making. Kadam features in Indian religions and mythologies[5-8].

## MATERIALS AND METHODS

### Plant material

Leaves and roots of *Neolamarckia cadamba* plants were collected from local areas of Berhampur, Odisha. The taxonomical identification of the plant specimen was done and voucher specimen was preserved in the Department of Pharmacognosy of the Royal College of Pharmacy and Health Sciences, Berhampur for further verification. The plant materials were air dried under shade, coarsely powdered and kept in airtight container until further use.

### Animals

Prior conducting the experiment, the ethical clearance for the study was granted by Institutional animal ethics committee (IAEC) of Royal College of Pharmacy and Health Sciences, Berhampur.

As per the OECD draft guidelines 423 received from CPCSEA, young female albino mice were used for acute toxicity

study. Whereas other in vivo methods were carried out by using Sprague-Dawley (SD) rats of both sexes. All the animals for the in vivo studies, with no prior drug treatment, were procured from the animal house of R.C.P.H.S., Berhampur and housed in polypropylene cages with clean sterilized husk bedding (six mice or three rats/ cage). Bedding was changed every alternate day to maintain proper hygienic condition. Animals were maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5°C) with a 12:12 hour light: dark cycle. The animals were fed with standard laboratory food diet made in-house recommended by National institute of nutrition (NIN), Hyderabad and pure drinking water *ad libitum*. The animals were acclimatized to laboratory hygienic conditions in the departmental laboratory for 7 days before commencing the experiment.

**Chemicals**

Glibenclamide was obtained from Dr. Reddy's Laboratories, Hyderabad. Blood glucose test-strips of Ascensia Entrust of Bayer Health Care and Diagnostic kits of Crest Biosystems, a division of Coral clinical systems, India were purchased. All other chemicals used for study were of analytical grade.

**Preparation of extracts**

Dried and coarsely powdered plant materials (400 gm of Leaves and roots of *Neolamarckia Cadamba*) were extracted separately by successive extraction process using soxhlet apparatus. Solvents were chosen depending upon their increase in polarity like Petroleum Ether (60-80°C) and ethanol. The extraction was carried out for 72 hours for each solvent. All the extracts were dried using rotary vacuum evaporator and freeze dryer. Their percentage yields were determined and stored in dessicator until further use.

**Phytochemical screening**

Different extracts obtained from the above extraction process i.e. Ethanolic Leaf Extract of *Neolamarckia Cadamba* (ELENC) and Ethanolic Root Extract of *Neolamarckia Cadamba* (ERENC) were analyzed for presence of various phytoconstituents such as alkaloids, glycosides, flavones, tannins, terpenes, sterols, saponins, fats and sugars by the method of qualitative phytochemical analysis.[9, 10, 11]

**Acute toxicity studies**

The acute oral toxicity studies of extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17th December 2001 received from CPCSEA, Ministry of social justice and

empowerment, Govt. of India. Administration of the stepwise doses of ethanolic extracts of *Neolamarckia cadamba* from 100 mg/kg up to the dose 2000 mg/kg to young female albino mice and observed the signs of toxicity up to 72 hr in the tested animals[12].

The female albino mice 25-30 gm were divided into different groups of six animals each. The control group received 10 ml/kg body weight of distilled water orally. The other groups received the ethanolic extracts of *Neolamarckia cadamba* at dose levels of 100, 500, 1000, 1500, 2000 mg/kg body weight through oral route. After administration of dose the animals were observed continuously for the first 4 hr and occasionally up to 24 hr and at the end of 72 hr[13] for recording mortality, if any. Additional observations like behavioral changes, somato motor activity, tremors, convulsions, tonic extension, strub tail, muscle spasm, loss of righting reflex, ataxia, sedation, hypnosis, lacrimation, diarrhea, salivation, writhing, changes in skin, fur, eyes, mucous membranes etc. were recorded. One tenth of upper limit dose and its half dose and double dose were selected as the levels for examination of therapeutic activity.

**Oral Glucose Tolerance Test**

After acclimation for 7 days, the oral glucose tolerance test was performed in overnight fasted normal rats[14]. All the rats were randomly divided into five groups (n=6) and administered different drugs as per the schedule given in Table 1.

The rats were fasted for 12h (free access to water) and administered the above drugs to respective groups. Zero minute blood sugar level was determined from overnight fasted animals. After 30 minutes of the drug treatment (p.o.) the rats of all groups were orally fed with glucose 4 gm/kg. Blood glucose concentration was determined after 30, 60, 90 and 120 minutes of glucose loading. The blood samples were collected from the tail tip and measured by using glucometer and blood glucose test-strips.

**Hypoglycemic activity**

The hypoglycemic activity was performed in overnight fasted normal rats as per the method described by Jarald *et al*., 2008. All the rats were randomly divided into five groups of six rats each and administered different drugs as the schedule given in Table 2.

The rats were fasted for 12h (free access to water) and administered the above drugs to respective groups. Zero minute blood sugar level was determined from overnight fasted animals i.e. before oral administration of drug. The blood glucose concentration was also measured after 30,

60 and 120 minutes of oral administration of drug. The blood samples were collected from the tail tip of the rats and measured the glucose concentration by using glucometer and blood glucose test-strips[14].

**Statistical analysis**

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

**RESULTS**

**Preliminary phytochemical screening**

The percentage yields of ELENC and ERENC were found to be 12.4% w/w and 9.7 % w/w respectively. Both extracts ELENC and ERENC contained carbohydrates, saponins, alkaloids, tannins, flavons and flavonoids. .

Several papers have recently reported the hypoglycemic and hypolipidemic effects of phenolic compounds such as flavonoids, flavons, tannins etc. The phytochemical studies of revealed that ELENC and ERENC has contains phenolic compounds such as flavons, flavonoids, tannins etc.

**Acute Toxicity Study of ELENC and ERENC**

In all the cases, no death was observed. Additional observations like changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern were also found to be normal. Attention was also given to observation of tremors and convulsions, were also absent in all groups. The results were recorded in table3.

**The effect of ELENC and ERENC in glucose loaded hyperglycemic animals**

The antihyperglycemic effect in glucose loaded hyperglycemic rats (shown in table-4) were studied after administration of ELENC and ERENC at the dose of 200 and 400 mg/kg and glibenclamide 5 mg/kg to respective groups. After 30 minutes of the glucose load, there was a significant rise in the blood glucose level of the control animals and at the end of two hours, the glucose level declined. The extract exhibited significant antihyperglycemic effect at 200 and 400 mg/kg dose levels after glucose load, compared to glibenclamide group animals.

**The effect of ELENC and ERENC in fasted normal rats**

The hypoglycemic effect in fasted normal rats were evaluated (shown in table-5), after administration of the ELENC and ERENC at the dose of 200 and 400 mg/kg; and glibenclamide 5 mg/kg to respective

group. After 30 min. of drug administration up to the end of 2 hours the blood glucose levels of the standard animals were declined. The extract show hypoglycemic activity at 200 and 400 mg/kg mg/kg dose.

## DISCUSSION

The present study was undertaken to examine the hypoglycemic activity of ethanolic leaf extract of *Neolamarckia cadamba* and ethanolic root extract of *Neolamarckia cadamba*. Antihyperglycemic effect was studied on glucose loaded rats and hypoglycemic effect was studied on the normal rats.

Effective blood glucose control is the key for preventing or reversing diabetic complications and improving the quality of life in patients with diabetes. Thus, sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications[19]. On the basis of this statement, we have selected the glucose induced hyperglycemic model to screen the antihyperglycemic activity of the plant extracts.

In the glucose loaded hyperglycemic model, the ELENC and ERENC tested for antihyperglycemic activity exhibited significant antihyperglycemic activity at the dose level of 200 and 400 mg/kg. Excessive amount of glucose in the blood induces the insulin secretion. This secreted insulin will stimulate peripheral glucose consumption and control the production of glucose through different mechanisms[20]. However, from the study (glucose control), it was clear that the secreted insulin requires more than 2 h to bring back the glucose level to normal. In case of the ELENC and ERENC; and drug treated groups, the glucose levels did not exceed the control group, giving an indication regarding the supportive action of the extracts and drug in the glucose utilization. The ELENC and ERENC, when tested for hypoglycemic activity, exhibited significant activity at the dose level of 200 and 400 mg/kg, suggesting its mechanism might be similar to sulfonylureas. Sulfonylureas increase insulin secretion by act on β-cells of islets of langerhens.

## CONCLUSION

The results obtained from the pharmacological screening conclude that the ethanolic leaf extract of *Neolamarckia cadamba* and ethanolic root extract of *Neolamarckia cadamba* has shown potential activity in decreasing the serum glucose level. This research supports the inclusion of this plant in traditional antidiabetic preparations.

**ACKNOWLEDGEMENTS**

The authors thank to Royal College of Pharmacy and Health Sciences, Berhampur, Odisha, for providing required facilities to carry out this research work.

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**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 (4 mg) |
| Group-III | Standard | Gliclazide (5 mg/kg) + Glucose (4 mg) |
| Group-IV | ELENC-200 | ELENC (200 mg/kg) + Glucose (4 mg) |
| Group-V | ELENC-400 | ELENC (400 mg/kg) + Glucose (4 mg) |
| Group-VI | ERENC-200 | ERENC (200 mg/kg) + Glucose (4 mg) |
| Group-VII | ERENC-400 | ERENC (400 mg/kg) + Glucose (4 mg) |

**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 | Gliclazide (5 mg/kg) |
| Group-III | ELENC-200 | ELENC (200 mg/kg) |
| Group-IV | ELENC -400 | ELENC (400 mg/kg) |
| Group-V | ERENC-200 | ERENC (200 mg/kg) |
| Group-VI | ERENC -400 | ERENC (400 mg/kg) |

**Table 3: Acute toxicity studies of Ethanolic extracts of Neolamarckia cadamba**

| Treatment | Dose (mg/kg) | No. of Mice | No. of Death | Signs of toxicity | LD50 |
|---|---|---|---|---|---|
| Control (Distilled water) | 10 ml/ kg | 6 | 0 | - | - |
| Ethanolic Leave extracts of Neolamarckia cadamba (ELENC) | 100 | 6 | 0 | - | > 2000 mg/kg |
| | 500 | 6 | 0 | - | |
| | 1000 | 6 | 0 | - | |
| | 1500 | 6 | 0 | - | |
| | 2000 | 6 | 0 | - | |
| Ethanolic Root extracts of Neolamarckia cadamba (ERENC) | 100 | 6 | 0 | - | > 2000 mg/kg |
| | 500 | 6 | 0 | - | |
| | 1000 | 6 | 0 | - | |
| | 1500 | 6 | 0 | - | |
| | 2000 | 6 | 0 | - | |

**Table 4: Effect of different extracts of ELENC and ERENC on blood glucose concentration in glucose loaded rats**

| Groups | Mean blood glucose concentration (mg/dl) at different time | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Normal control | 85.47 ± 1.24 | 84.56 ± 1.63 | 87.29 ± 2.45 | 81.46 ± 2.74 | 86.38 ± 2.03 |
| Glucose loaded | 87.91 ± 1.56 | 148.74 ± 1.26$^{a}$ | 155.98 ± 2.82$^{a}$ | 159.21 ± 1.92$^{a}$ | 164.85 ± 2.62$^{a}$ |
| Control | | | | | |
| Standard | 84.69 ± 1.48 | 109.37 ± 1.01* | 96.81 ± 1.71* | 86.63 ± 1.82* | 75.24 ± 1.27* |
| ELENC-200 | 87.69 ± 2.34 | 104.07 ± 2.75* | 112.73 ± 2.91* | 102.34 ± 3.53* | 94.15 ± 3.47* |

| | | | | | |
|---|---|---|---|---|---|
| ELENC-400 | 85.52 ± 2.83 | 118.31 ± 2.73* | 105.96 ± 3.02* | #93.46 ± 2.95* | #82.64 ± 2.56* |
| ERENC-200 | 86.25 ± 0.76 | 115.47 ± 0.32* | 102.57 ± 0.95* | 90.08 ± 1.52* | 85.87 ± 2.64* |
| ERENC-400 | 84.28 ± 1.28 | 108.47 ± 1.49* | 93.67 ± 2.61* | #84.60 ± 1.35* | #74.38 ± 2.18* |

*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 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.*

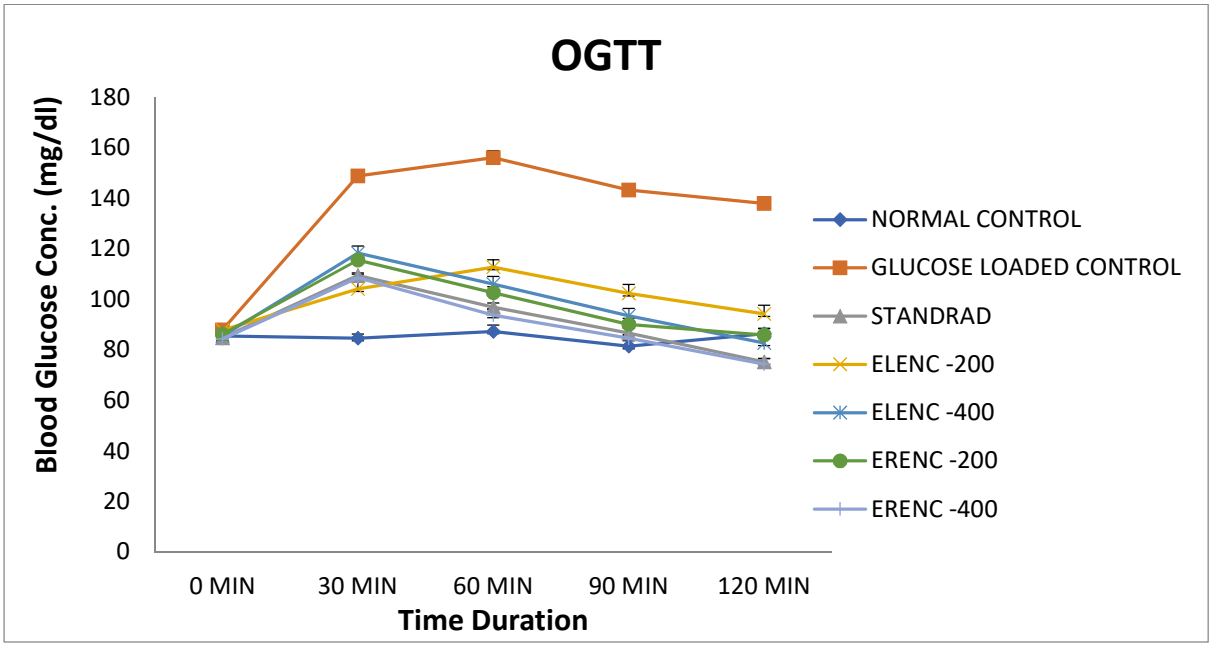


**Figure 1: Percentage change in blood glucose concentration of different groups of OGTT Rats**

**Table 5: Percentage change in blood glucose concentration of different groups of OGTT Rats**

| Groups | Percentage changes in blood glucose (mg/dl) at different time | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Standard | 3.66 | 26.46 | 37.93 | 39.50841 | 45.41893 |
| ELENC-200 | 0 | 30.03 | 27.72 | 28.53851 | 31.70112 |
| ELENC-400 | 2.71 | 20.45 | 32.06 | 34.73919 | 40.05078 |
| ERENC-200 | 1.88 | 22.36 | 34.24 | 37.09936 | 37.70765 |
| ERENC-400 | 4.12 | 27.07 | 39.94 | 40.92591 | 46.0428 |

**Table 6: Effect of different extracts of ELENC and ERENC 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 | 82.83 ± 0.477 | 85 ± 0.816 | 83.5 ± 0.76 | 83.83 ± 1.01 | 84.33 ± 0.42 |
| Standard | 82.5 ± 0.7638 | 64.16 ± 0.7631* | 52.83 ± 0.94* | 44.33 ± 0.88* | 35 ± 0.57* |
| ELENC-200 | 83 ± 0.73 | 80.16 ± 0.6009 | 79.83 ± 0.6009 | 75.16 ± 0.792* | 71.5 ± 0.67* |
| ELENC-400 | 83.5 ± 0.76 | 77.16 ± 0.60* | #65.5 ± 0.42* | #60.66 ± 0.49* | #54 ± 0.96* |
| ERENC-200 | 82.33 ± 1.05 | 79.66 ± 0.66 | 70.16 ± 0.79* | #65.16 ± 0.60* | #61.5 ± 0.76* |
| ERENC-400 | 79.5 ± 0.56 | 71.33 ± 0.88* | #63 ± 0.57* | #55.5 ± 0.42* | #45.5 ± 0.76* |

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

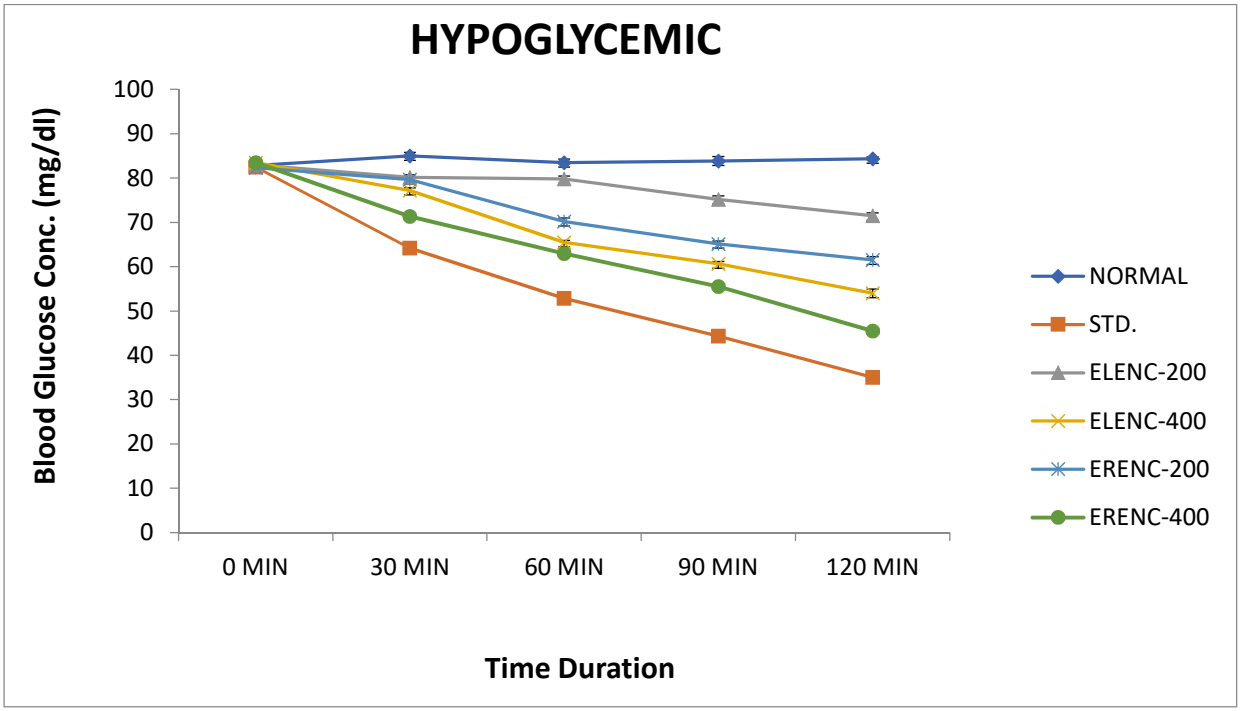


**Figure 2: Percentage change in blood glucose concentration of different groups of hypoglycemic rats**

**Table 7: Percentage change in blood glucose concentration of different groups of hypoglycemic rats**

| Groups | Percentage change in blood glucose conc. at different time | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Standard | 0 | 24.5098 | 36.72655 | 47.1173 | 58.49802 |
| ELENC-200 | 0 | 5.686275 | 4.391218 | 10.33797 | 15.21739 |
| ELENC-400 | 0 | 9.215686 | 21.55689 | 27.63419 | 35.96838 |
| ERENC-200 | 0 | 6.27451 | 15.96806 | 22.2664 | 27.0751 |
| ERENC-400 | 0 | 16.07843 | 24.5509 | 33.79722 | 46.04743 |