



## Evaluation Of Hypoglycemic & Anti-Hyperglycemic Activity Of Aqueous Extract Of *Leucas indica* (L)R.Br.var.nagulapuamiana Against Streptozotocin Induced Diabetes In Rats

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### ABSTRACT

#### Keywords:

Hypoglycaemic,  
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Induced Diabetes,  
Rats

The pharmacological work is to explore the hypoglycaemic and antihyperglycaemic activity of aqueous extract of root of *Leucas Indica* herb. The extract was evaluated for its specific physical and chemical characteristics in order to standardize it. The aqueous extract of root of *Leucas Indica* was tested at 3 dose levels (100, 200 and 400 mg/kg each) in rats. In each case the initial test was performed at dose of 100 mg/kg. The actions were compared with standard Tolbutamide drug at a dose of 40 mg/kg. The data obtained was analyzed with the one-way analysis of variance (ANOVA), followed by a post hoc of Dunnett's T-test. The study was revealed that *Leucas Indica* was found to have hypoglycemic and antihyperglycaemic activities similar to tolbutamide.



## 1. Introduction

As per the World Health Organization more than 176 millions of patients are suffering from diabetes mellitus type -II globally, hence it is a global health problem (WHO 2012)<sup>1</sup>. India has become more than 63 millions of diabetic patients (IDF 2012)<sup>2</sup> Diabetes mellitus is metabolic disorder with loss of glucose homeostasis and disturbances of carbohydrate, fat, and protein metabolism and represented by hyperglycemia, lipidaemia and oxidative stress and slowly affects kidneys, eyes, skin, heart and blood vessels (K.Imam, 2012 & A Elost 2012)<sup>3,4</sup>. Diabetes causes chronic morbidity and disability. Type 2 diabetes mellitus initially appears to be insulin resistance for the period with increase in insulin secretion from pancreatic gland and as disorder advances, the pancreatic functions are decreased and failed to meet peripheral requirements (Inzucchi 2003)<sup>5</sup>. Diabetic Mellitus produces major complications such as atherosclerosis of heart,

myocardial infarction, heart failure, predisposition to infection, decreased joint mobility, cataract development and hardening of skin (Knentz AJ & Natras M 1991)<sup>6</sup>.

The count of diabetic patients are increasing day by day in India may be because of the change in the life style and intake of fast food (Devi Manickam)<sup>7</sup>. It is tough job to control diabetes and anti-diabetic agents are reported with adverse effects like hypoglycaemic coma, hypersensitivity, metallic taste and insulin resistance (Nyemb Nyunai AB)<sup>8</sup>. The herbal compounds are gaining popularity across the globe in recent past due to their low adverse effects (Avinash Patil A et al 2013)<sup>9</sup>.

According to ethanobotanical information, around 800 medicinal plants are reported with hypoglycaemic & anti-hyperglycemic activities (Sweety Lanjhiyana et al 2011)<sup>10</sup>. India is hub of herbal plants and hence our current



research work is planned to find out safe and effective anti-diabetic agents (Avinash Patil A et al 2013)<sup>9</sup>. Herbal medicines are popular in India than allopathic for various disorders.

*Leucas indica*(L)R.Br.var.nagulapuamiana belongs to Lamiaceae family commonly called as Gum, Tumba & dandokalos which is distributed all over India along road side waste land, river banks and rocky areas<sup>11, 12</sup>. It is an erect herb with pubescent branching. The leaves are linear lanceolate in nature while the flowers are white with four stamens. Traditionally, it is used in Garhwal region of Uttarakhand as a wound healer. The leaves of this plant are squeezed and placed on wounds to obtain wound healing<sup>13</sup>. Leaves are also used as vermifuge, stomachic, sedative and in sores. This plant is widely used in psoriasis, chronic skin eruptions and painful<sup>14,15</sup>. The herb is also used in jaundice, inflammation, asthma, dyspepsia, fever and cold, snake bites and

scorpion stings<sup>16,17</sup>. The phytochemicals like phenylethanoid, glycosides were isolated from the aerial parts of *Leucas indica* having antioxidant property<sup>11, 12</sup>.

Chemical analysis of various products of the plant showed the presence of carbohydrates, alkaloids, steroids, flavonoids, Triterpenoids, Fatty alcohol etc. A new phenylpropanoid glycoside was reported by Mostafa et al., 2014<sup>18</sup>. During their work they found  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)-1-O-caffeoyl- $\beta$ -D-glucopyranoside (1) along with two known phenylethanoid glycosides,  $\beta$ -(3-hydroxy-4-methoxy-phenyl)-ethyl-O-( $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-O-( $\alpha$ -L-rhamnosyl) (1 $\rightarrow$ 3)-6-O-E-feruloyl- $\beta$ -D-glucoside(2)(incanoside A) and  $\beta$ -(3,4-dihydroxy-phenyl)-ethyl-O-( $\alpha$ -L-rhamnosyl)-(1 $\rightarrow$ 3) O-( $\beta$ -D-glucoside)-(1 $\rightarrow$ 6)-4-O-E-caffeoyl- $\beta$ -D-glucoside (3). The detailed account on the chemistry of essential oil isolated from *Leucas Indica*



was given by Rosamma (2002). During the study he found that the characteristic odour of the plant is due to the presence of hexane and octane derivatives. Moreover, the main aroma in the fresh plant is due to the presence of 1-octen-3-ol<sup>19</sup>.

The plant has been reported with Anthelmintic property<sup>20</sup> Anti-inflammatory activity<sup>21, 22</sup> Antimicrobial activity<sup>23</sup> Antioxidant activity<sup>24,25</sup> Antiulcer properties<sup>26</sup> Hepatoprotective activity<sup>27</sup> and Anxiolytic activity<sup>28, 29</sup>.

The work has been planned to evaluate hypoglycaemic & anti-hyperglycaemic activities of *L.indica*(L) *R.Br.var.nagulapuamiana* in rats.

## 2. MATERIALS AND METHODS

**Preparation Of Aqueous Extract:** The plant material was powdered and macerated with chloroform: water (1:9) for seven days in glass container. Chloroform-water was served as

antimicrobial agent in the extract. As per the procedure, the extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites (Harborne JB 1973)<sup>30</sup>.

**Acute Toxicity Studies:** The acute toxicity studies were conducted for extract in mice at limit test dose of 2000 mg/kg according to OECD 423 guidelines. The mice were fasted and administered the plant extract as per the procedure and observed for mortality for 24 hrs and found no death. (Kandelwal KR 2000, Bugar GT & Miller CL 1989).<sup>31, 32</sup>

**Preliminary Qualitative Phytochemical Tests:** The aqueous extract of *L.indica* was subjected for qualitative chemical tests to find out the functional groups presence such as sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannis, proteins and phenolic steroids (Kokate CK 1994)<sup>33</sup>.



### Hypoglycemic And Antihyperglycaemic

**Activities Of Leucas Indica:** The hypoglycemic and antihyperglycaemic activities of aqueous extract of leaves of *L.indica* were studied by estimating glucose levels in blood. The three doses of 100, 200 and 400 mg/kg of extract were selected for this research in normal and diabetic models. The animals are divided into groups, each consisting of six animals. The suspending agent is 1% Sod. CMC. Zero hour blood glucose levels are estimated and served as control or normal glucose levels in all groups.

The extract of three doses of extract as per experiment is administered and blood samples are collected & analyzed for blood glucose levels. Tolbutamide is employed as standard drug.

**Experiment:** The albino rats (150-250g) were randomly selected and divided into ten groups, each consists of six animals. They were maintained under standard conditions (Room temperature at 25±20C,

12 h light/dark and free access to food along with water up to two weeks before the experiment to adapt to laboratory conditions). The animals were deprived of food for 18 hour and water allowed ad libitum prior to experiment<sup>34</sup>.

Group I: Received vehicle for 24 hours (Normal)

Group II: Diabetic rats (STZ, 45 mg/kg i.p) received 1% Sod.CMC orally

Group III: Normal Rats received Tolbutamide 40mg/kg p.o.(Normal)

Group IV: Normal Rats Received *L.indica* 100 mg/kg p.o. (Normal)

Group V: Normal Rats Received *L.indica* 200 mg/kg p.o. (Normal)

Group VI: Normal Rats Received *L.indica* 400 mg/kg p.o. (Normal)

Group VII: Diabetic Rats Received Tolbutamide 40 mg/kg p.o. (Diabetic)



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Group VIII: Diabetic Rats Received L.indica 100 mg/kg p.o. (Diabetic)      Group X: Diabetic Rats Received L.indica 400 mg/kg p.o (Diabetic)

Group IX: Diabetic Rats Received L.indica 200 mg/kg p.o. (Diabetic)

Table.1.The hypoglycemic activity of aqueous extract of L.indica in normal Rats

S.No	Groups	The percentage of reduction of blood Glucose levels mg/dL (hr)						
		1	2	4	6	8	16	24
1	Normal	2.03	1.80	1.85	5.21	6.69	8.44	9.50
2	Tolbutamide	8.08	14.13	23.98	34.66**	27.39	18.14	13.67
3	<i>L.indica</i> 100 mg/kg	1.36	4.70	6.03	6.47	5.21	3.61	2.22
4	<i>L.indica</i> 200 mg/kg	2.96	6.41	10.96	12.94**	8.72	5.13	2.46
5	<i>L.indica</i> 400 mg/kg	6.18	13.36	21.85	30.56**	21.98	11.30	2.75

Values are expressed in Mean±SEM, n=6, P<0.05\*, 0.01\*\*, 0.001\*\*\* Statistical analysis by paired 't' test.



**Table.2. The Anti-hyperglycemic activity of aqueous extract of *L.indica* in Diabetic Rats**

S.No	Groups	The percentage of reduction of blood Glucose levels mg/dL (hr)						
		1	2	4	6	8	16	24
1	Diabetic	2.28	3.58	5.62	5.36	7.66	8.16	5.07
2	Tolbutamide	12.32	16.99	35.34	46.28**	36.99	20.31	12.12
3	<i>L.indica</i> 100 mg/kg	02.51	05.71	09.19	12.35**	09.81	07.02	06.98
4	<i>L.indica</i> 200 mg/kg	03.94	08.35	14.47	19.41**	16.88	15.01	13.04
5	<i>L.indica</i> 400 mg/kg	06.45	12.98	18.85	24.66**	22.77	20.76	19.24

Values are expressed in Mean±SEM, n=6, P<0.05\*, 0.01\*\*, 0.001\*\*\* Statistical analysis by paired 't' test.

The aqueous extract of *L.indica* at a dose of 100 mg/kg was administered to animals in Group IV (Normal) and exhibited maximum decrease in blood glucose levels at 6 hours (81.68±1.63 mg/dl). The percentage reduction in blood glucose was found to be 6.47%. The results clearly confirmed that the extract of *L.indica* possess hypoglycaemic activity. The aqueous extract of *L.indica* at a dose of 200 & 400 mg/kg were administered to animals in Group V & VI (Normal)

respectively. The animals in Group in V have shown maximum decrease in blood glucose levels at 6 hrs (74.27±0.95 mg/dl). The percentage of blood glucose levels reduction at 6 hrs was recorded as 18.44%.

The blood glucose levels of Group VI which received the extract at a dose of 400 mg/kg at 6 hrs were found to be 66.03±0.60 mg/dl and the decrease in percentage of blood glucose levels was found to be 25.42% in normal rats. The results clearly mention that the plant extract possess promised hypoglycaemic activity in normal rats.



The diabetic animals in group VII were administered with aqueous extract of root of *L.indica* at a dose of 100 mg/kg and the maximum decrease in blood glucose levels at 6 hr ( $240.47 \pm 0.32$  mg/dl) and percentage reduction in blood glucose levels found to be 12.68 %. The diabetic rats in Group VIII & IX were administered with at a dose of 200 & 400 mg/kg of *L.indica* extract. The maximum reduction in blood glucose levels in group VIII & IX were found to be  $202.96 \pm 1.91$  mg/dl &  $171.80 \pm 2.37$  mg/dl respectively and the decrease in percentage of blood glucose levels found to be 25.41% & 37.76% respectively in group VII & IX against streptozotocin induced diabetes.

### 3. Conclusion:

The phytochemical investigation of the aqueous extract of root of *L.indica* (*L.* *R.Br.var.nagulapuamiana*) has revealed the presence of secondary metabolites such as flavonoids, phenolic compounds, glycosides, sterols, phenolic steroids & carbohydrates. The acute toxicity study of plant extract at a limit test dose of 200 mg/kg exhibited no mortality & no morbidity. The rigorous study

of blood glucose levels in animals has confirmed a promised hypoglycemic & anti-hyperglycemic activity.

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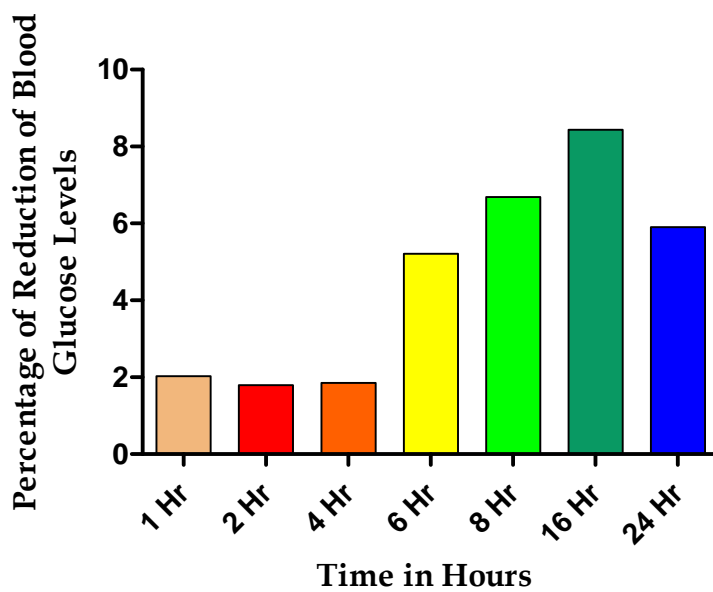
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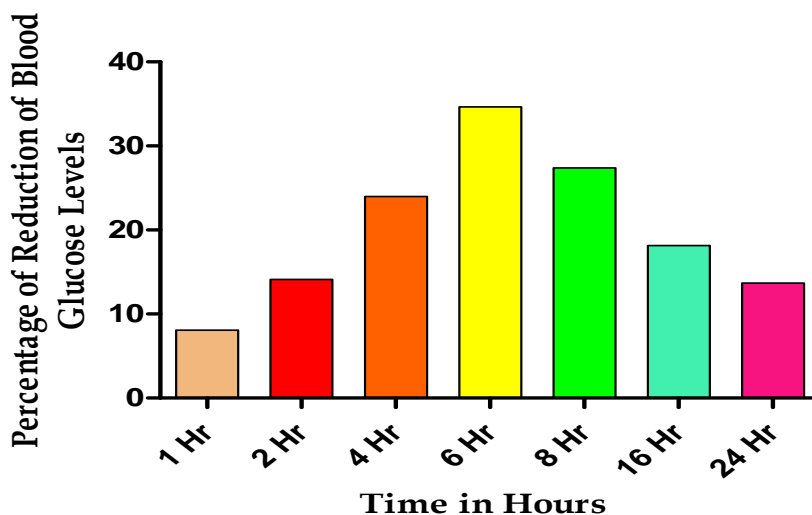
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Effect of Sod. CMC on Blood Glucose levels of Normal Rats



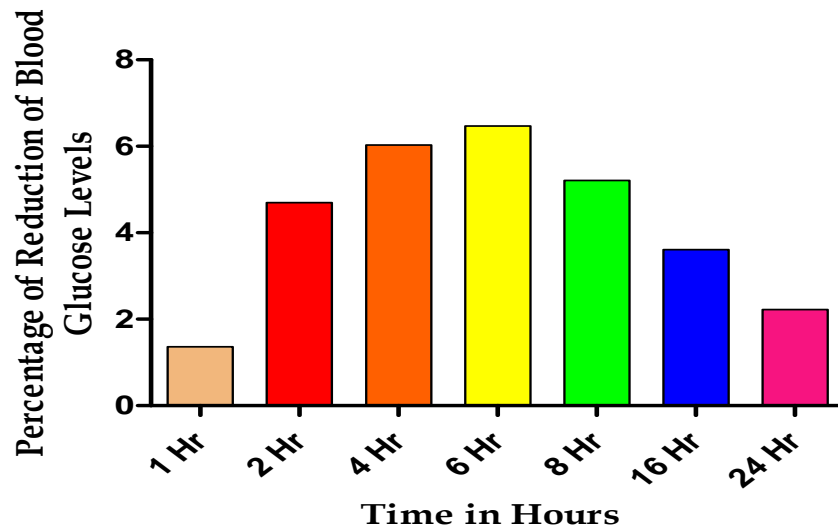
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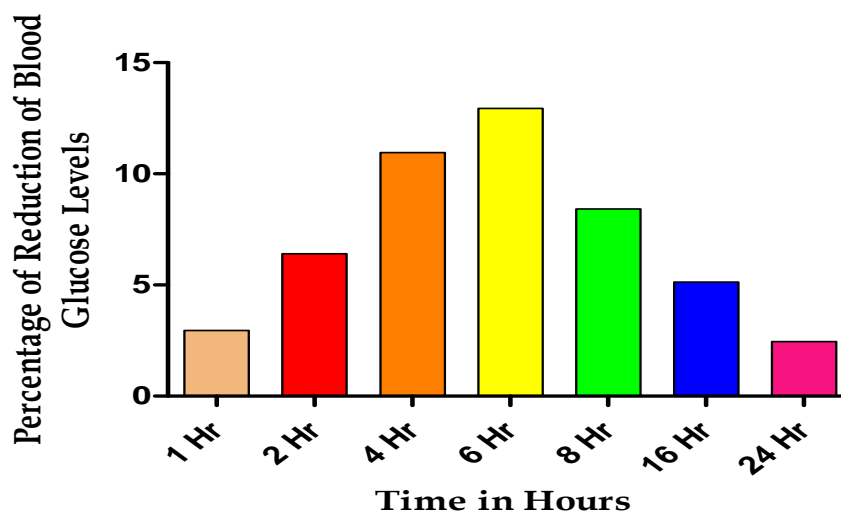


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Effect of Aqueous Extract of Roots of *L.indica* (100 mg/kg) on Blood Glucose Levels in Normal Rats

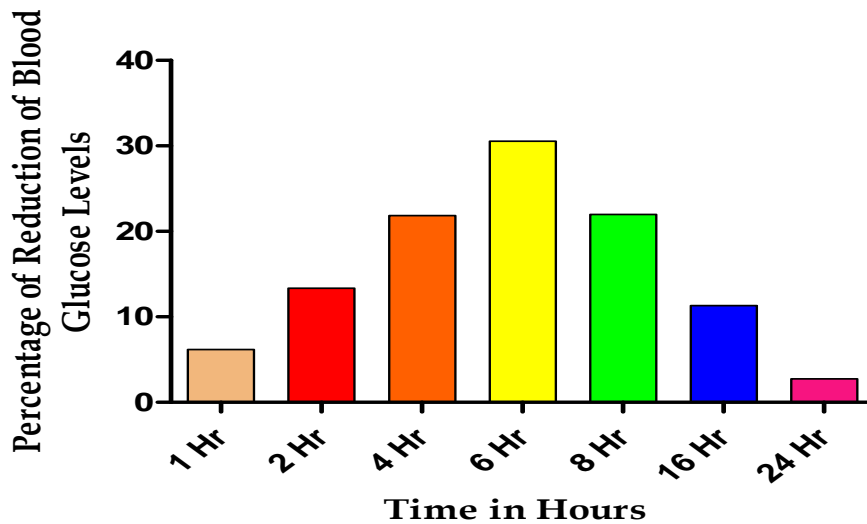


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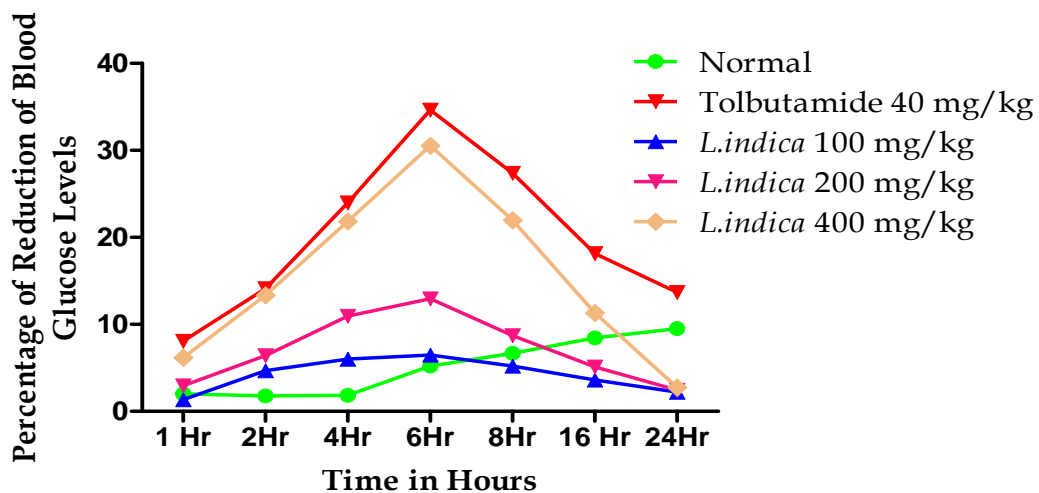




Effect of Aqueous Extract of Roots of *L.indica* (400 mg/kg) on Blood Glucose Levels in Normal Rats



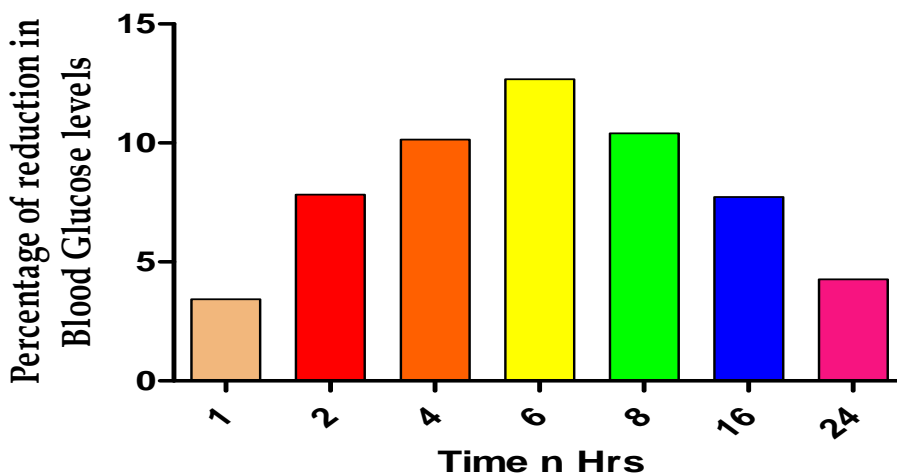
Changes of Blood Glucose levels with different treatments in Normal Rats



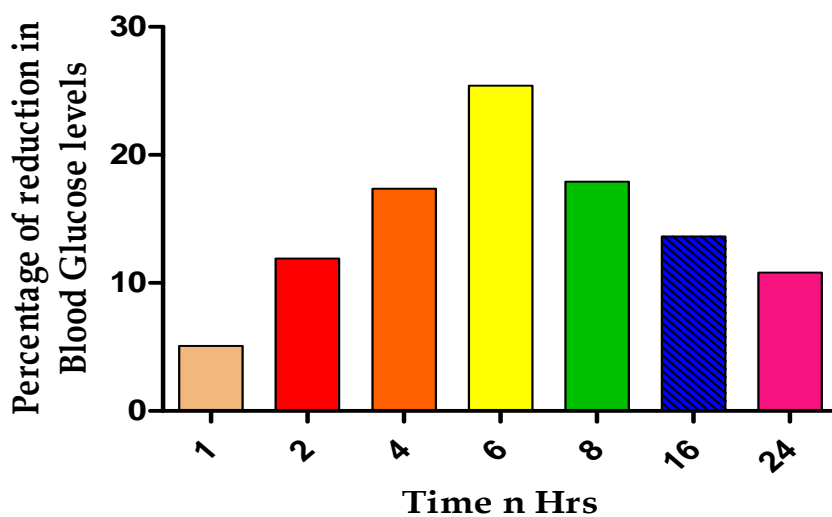
Effect of Aqueous Extract of Roots of *L.indica* (100 mg/kg) on Blood Glucose Levels in



Diabetic Rats

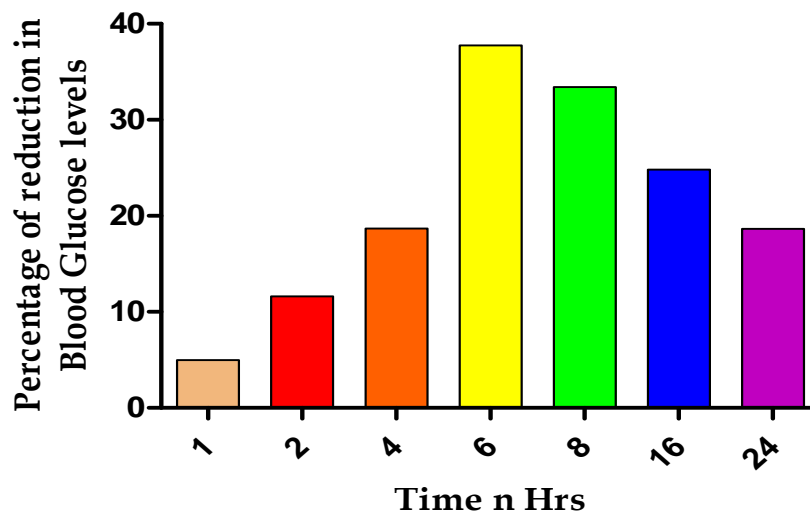


Effect of Aqueous Extract of Roots of *L.indica* (200 mg/kg) on Blood Glucose Levels in Diabetic Rats





Effect of Aqueous Extract of Roots of *L.indica* (400 mg/kg) on Blood Glucose Levels in Diabetic Rats



Changes of Blood Glucose levels with different treatments in Diabetic Rats

