



Anti-Diabetic Activity of *Ochna Obtusata* Leaves In Alloxan Induced Rats

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ABSTRACT

This study was conducted to investigate the anti-diabetic effect of ethnoc extract of *Ochna obtusata* leaves in Alloxan induced diabetic Wistar albino rats. The anti-diabetic activity was assessed by determining and comparing the fasted blood glucose level in the test group (200,400 mg/kg) with that of the diabetic control group and glibenclamide 20mg/kg was used as a reference standard. In the alloxan-induced diabetic rats *Ochna obtusata* produced a significant reduction in fasting blood glucose levels. It acts in a dose dependant manner to bring back the raised blood sugar levels to normal. The 400mg/kg treated group has showed maximum reduction in blood glucose (148.26 mg/dl) which is closer to that of standard Glibenclamide (145.71 mg/dl). We can conclude that ethnoc extract of *Ochna obtusata* is capable of managing hyperglycemia induced by alloxan. The extract seems to be one of the potential sources for the development of a novel phytotherapy for diabetes mellitus.

Keywords: Anti diabetic activity, *Ochna obtusata*, Alloxan induced diabetis , Hypoglycemic activity.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from impaired secretion of insulin or insulin resistance. When the amount of blood sugar increases, it leads to disruption in the metabolism of carbohydrates, proteins and fats¹. Diabetes can lead to diabetic retinopathy, diabetic nephropathy and diabetic neuropathy. It doubles the risk of vascular diseases such as atherosclerosis, angina and myocardial infarction².

The prevalence of diabetes mellitus is expected to rise up to 4.4% in the world by 2030³. Being a multifactorial disease it is becoming the third killer of the mankind and continue to be a serious challenge to international public health in the world today⁴. Unfortunately conventional or synthetic drugs used in the treatment of diabetes are inadequate and sometimes can have serious side effects. Hence, the research for ideal drugs still continues and has been extended to herbal drugs; About 800 herbal plants with antidiabetic activity are present all over the India⁵. Among those 150 medicinal plants are reported to possess diabetic activity⁶. Therefore many studies have confirmed the herbal plants benefits with antidiabetic activity by beta-cell restorative properties, Modulating glycolysis, gluconeogenesis, Krebs cycle, and enhancing insulin level, mimicking the action of insulin^{7,8}.

Ochna obtusata (Ochnaceae) commonly called as Tammi, panjaram, Ramatana Champaka or Golden Champak is one such medicinal plant possesses several pharmacological properties, where, few are already reported and few are yet to be investigated. It occurs throughout India, most commonly grow in forests⁹.

Stem bark of this plant appears to be thick, smooth grayish from the outside and pink when stem is peeled off. The stem juice is commonly used to treat gastrointestinal disorders in Tanzania¹⁰. Leaves simple, alternate, distichous; stipules caducous and leaving scar; petioles ca. 0.4 cm long, planoconvex, glabrous; lamina 16 x 5 cm, elliptic or elliptic-oblong to obovate, apex acute to rounded, base acute to rounded, margin serrate, shining above, chartaceous, glabrous beneath; midrib raised above; secondary nerves ca. 12 pairs, ascending towards apex; tertiary nerves slender, reticulo-percurrent. The leaves and roots of this plant is used to treat the asthma, bronchitis¹¹. Recently, it has been proved for ulcer treatment and claimed to possess diuretic properties^{12,13}.

To best of our knowledge, no other biochemical investigations had been carried out on anti diabetic activity of *Ochna obtusata*. So the present investigation was carried out to study the anti diabetic activity of *Ochna obtusata* leaves extract in Alloxan induced diabetic rats.

MATERIAL AND METHODS

Plant material

The leaves of the plant were collected in the month of July, 2013 from Badrachalam Forest, Khammam, Andhra Pradesh. The plant was identified and authenticated. A Herbarium specimen of the plant was preserved in the Department of Pharmacognosy of our institute for further reference.

Preparation of extracts

The leaves of the plant were shade dried, powdered mechanically and subjected to Soxhlet extraction successively with ethanol. After extraction the filtrate was concentrated on a rotary evaporator under vacuum at 20°C till a residual mass was obtained. Ten grams of the dried extract was triturated with 9 mL of vehicle. Vehicle was then added gradually until the final concentration of the extract was 10%.

Phytochemical Screening of extract

The ethanolic extract of *Ochna obtusata* leaves were subjected to preliminary phytochemical screening for their presence or absence of active phytochemical constituents.

Experimental animals

Healthy male Wistar albino rats at 8 weeks of age were purchased from Ghosh Enterprises, Kolkata. They were housed in a room, maintained at approximately 25 ± 2 °C. The photoperiod was 12 hrs light and 12 hrs dark cycle. For feeding, the rodent laboratory diet used which is supplied from Ryan Biotechnology Pvt Ltd, Hyderabad. The study was performed following approval from Institutional Animal Ethical Committee (IAEC) of Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak. (1358/ac/10/CPCSEA). Ethical norms were strictly followed during all experimental procedures.

Acute toxicity study

Acute toxicity studies were carried out using acute toxic class limit test dose guidelines 423 of Organization for Economic Co-operation and Development (OECD) ¹⁴. Acute toxicity of the test drug was carried out, using groups of three Wistar albino rats; by administering a dose of 2000 mg/kg body weight, p.o., and the control group received normal saline, and observed all the animals closely for 24 hrs for any mortality. It was observed that the ethanolic extract of *Ochna obtusata* was not lethal to the rats even at 2000mg/kg dose. Hence 200mg/kg and 400mg/kg taken as effective dose for further study.

Preparation of doses

All the extracts (200mg/kg, 400 mg/kg) were suspended in 2% v/w aqueous solution of tween 80 and glibenclamide (10mg/kg b.w.) in normal saline used as standard drug. The control group received normal saline orally.

Induction of diabetes

Over-night fasted rats were made diabetic by using Alloxan mono hydrate (120mg/kg body weight) dissolved in normal saline given intra peritoneally.

Anti diabetic Activity:

The Anti diabetic activity of the plant extracts was tested using alloxan induced model. The method used for evaluation of anti-diabetic was according to Jarald EE et al.; with some minor modifications¹⁵. Over-night fasted rats were made diabetic by using Alloxan mono hydrate (120mg/kg body weight, I.P) .The rats were then maintained for the next 24 h on 5% glucose solution in to prevent hypoglycemia. Three days later blood samples were withdrawn and used for the estimation of glucose levels. The diabetic rats (fasting blood glucose > 250 mg/dl) were selected and used for the study.

The diabetic rats were divided into four groups, each containing six animals.

Group I: The animals of this group served as diabetic control received alloxan mono hydrate.

Group II: The animals received extract of *Ochna obtusata* 200mg/kg daily for 7 days

Group III: The animals received extract of *Ochna obtusata* 400mg/kg daily for 7 days

Group IV: The animals received glibenclamide 10mg/kg b.w daily for 7 days .

Blood samples were collected from the tail vein used for the determination of fasting blood glucose at 0, 2, 4, and 6 h after treatment. The effect of *Ochna obtusata* extract was also tested for a prolonged treatment. Treatment was continued for 7 days. The fasting blood glucose levels were estimated on days 0, 1, 3, 5 and 7.¹⁶

Statistical analysis

The data are presented as mean \pm SEM and analyzed by one- way ANOVA,¹⁷ followed by Dunnett‘t’ test¹⁸. The results of all the extracts including the standard drug are compared with the result produced by control. And it is considered as significant as $P < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of *Ochna obtusata* extract revealed the presence of terpenoids, steroids, flavonoids, glycosides, alkaloids saponins and coumarin .

Acute toxicity

The extracts were found to be safe in the dose used and there was no mortality up to a dose of 2000 mg/kg body weight. It was found that even at 2000mg/kg dose did not manifest any significant abnormal signs, mortality, behavioral changes, and body weight changes. Toxicity signs such as tremors, salivation, diarrhea and lethargy were not observed. Confirming practically these are not has toxic activity.

Anti diabetic activity

The effect of ethanol extract of *Ochna obtusata* leaves are shown in (Table 1,2). Acute and subacute treatment of the *Ochna obtusata* extract in alloxan-induced diabetic rats resulted in a significant ($P<0.05$) decrease in the elevated blood glucose levels as compared to the control. In alloxan induced diabetic rats acute treatment of *Ochna obtusata* at a dose of 200mg/kg couldnot bring back the raised blood sugar levels to normal . But *Ochna obtusata* at a dose of 400mg/kg shown considerable reduction in blood glucose levels.

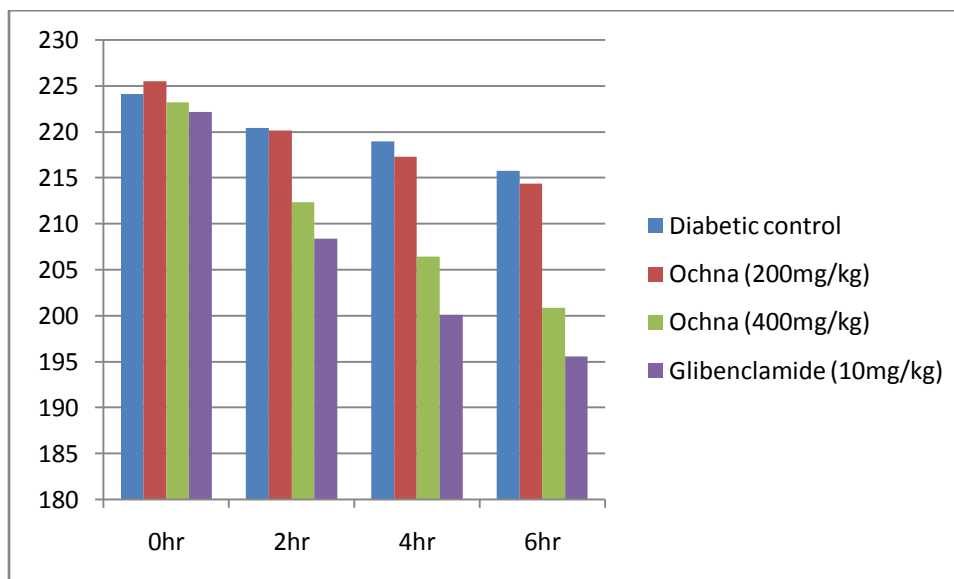
In subacute studies On prolonged treatment with of *Ochna obtusata* produced Significant reduction in the blood glucose levels. Both the doses of *Ochna obtusata* have shown significant ($p<0.05$) reduction of blood glucose. However the 400mg/kg treated group has shown maximum reduction (148.26 mg/dl on 7th day) which is closer to that of standard drug glibenclamide (145.71 mg/dl on 7th day).

Tabel-1: Effect of *Ochna obtusata* leaves extract in alloxan induced diabetic rats (Acute studies)

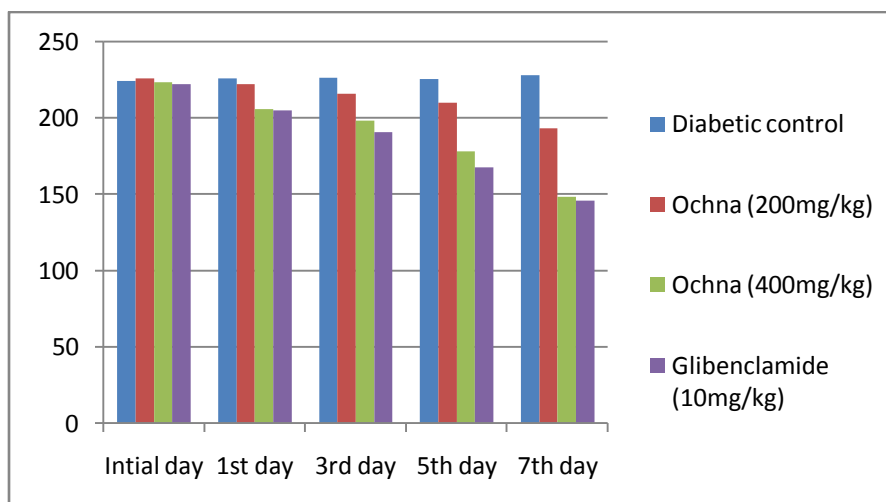
Group	Treatment	Blood glucose level (mg/dl)			
		0hr	2hr	4hr	6hr
I	Diabetic control	224.12±1.30	220.39±1.62	218.97±1.83	215.72±1.91
II	Ochna (200mg/kg)	225.51±1.48	220.13±1.76	217.26±1.35	214.32±1.31
III	Ochna (400mg/kg)	223.19±1.51	212.37±1.28	206.45±1.28	200.87±1.38
IV	Glibenclamide (10mg/kg)	222.15±1.27	208.34±1.81	200.12±1.71	195.61±1.58

Table 2: Effect of *Ochna obtusata* leaves extract in alloxan induced diabetic rats (Sub acute studies)

Group	Treatment	Blood glucose level (mg/dl)				
		Initial day	1 st day	3 rd day	5 th day	7 th day
I	Diabetic control	224.12±1.30	225.89±1.57	226.13±1.40	225.27±1.39	227.61±1.42
II	Ochna(200mg/kg)	225.51±1.48	222.14±1.42	215.81±1.32	209.76±1.54	193.14±1.75
III	Ochna(400mg/kg)	223.19±1.51	205.71±1.63	198.11±1.51	178.14±1.71	148.26±1.41
IV	Glibenclamide	222.15±1.27	204.81±1.87	190.34±1.83	167.34±1.52	145.71±1.24



Graph 1: Effect of *Ochna obtusata* leaves extract in alloxan induced diabetic rats (Acute studies)



Graph-2: Effect of *Ochna obtusata* leaves extract in alloxan induced diabetic rats (Sub acute studies)

CONCLUSION

The present study reveals the antidiabetic activities of *Ochna obtusata* extract in experimental animal model against alloxan induced hyperglycemia which was proved by reduced glucose levels. Further study is in progress to isolate the exact chemical constituent (s) and mechanism (s) of action.

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