ANTI-DIABETIC ACTIVITY OF *BUTEA MONOSPERMA* LAM TAUB FLOWERS AND ITS ISOLATED STEROLS IN ALLOXAN INDUCED DIABETIC RATS

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Summary

The effects of different extracts of *Butea monosperma* Lam Taub flowers and isolated sterols of sterol A and sterol B of petroleum ether ($40^{o} - 60^{o}$) extract on blood glucose level in normal and Alloxan induced rats was evaluated in the present study. Among five extracts (Petroleum ether, chloroform, ethyl acetate, alcohol and aqueous) all the extracts showed significant activity except chloroform extract in prolonged treatment. Isolated sterols of sterol A and sterol B from petroleum ether ($40^{o} - 60^{o}$) extract also showed significant reduction of blood glucose level in acute study.

Key Words: Alloxan, Anti-diabetic activity, *Butea Monosperma*.

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Introduction

Butea monosperma Lam Taub (Leguminaceae) is distributed through out India, in deciduous forest area upto 1200 m elevation [1]. Almost all parts of this plant including Flowers, Gum, Seeds, Leaves and its Bark possess important medicinal properties like anti-estrogenic [2], hepatoprotective [3], anticonvulsant [4], anthelmentic [5] and ocular anti-inflammatory [6] activities.

Traditionally flowers are used in diabetes [7], gout, herpes and in birth control. Palashapushpadi churna, the formulation containing *Butea monosperma* flower is one of the main ingredient that has shown significant activity in madhumeha roga as per clinical studies [8].

Materials and methods

Plant material

The flowers of *Butea monosperma* were collected from Jamboti ghat (Western ghat area) Karnataka, India in the month of February 2003 and authenticated by Prof. A. V. Kulkarni at the department of Botany G. S. Science Institute, Belgaum, Karnataka, India. A voucher specimen (B.M./FL/2005) is being maintained in the Department of Pharmacognosy and Phytochemistry, K. L. E. S's College of Pharmacy, Belgaum, Karnataka, India.

Preparation of Extracts

Shade dried powder of *Butea monosperma* flowers were subjected to exhaustive extraction by Soxhlet apparatus, successively with petroleum ether (40^{o} – 60^{o}), chloroform, ethylacetate, alcohol and aqueous. The extracts were filtered and concentrated at room temperature to avoid the decomposition of the natural metabolites. The dried extracts were stored carefully for preliminary phytochemical investigation and for animal study.

Preliminary Phytochemical Screening.

Preliminary phytochemical screening of extracts revealed that sterols and lipids were present in petroleum ether extract. Sterols and lipids were present in chloroform extract. Flavanoids, oils and lipids in ethylacetate extract. Triterpinoides, flavanoids lipids in alcoholic extracts. Carbohydrates, proteins, aminoacids, and phenolic compounds were present in the aqueous extract.

Isolation of sterols

The column chromatography was developed by Silica gel–G using graded solvent mixture of petroleum ether:acetone (95:5) yielded two major crystalline sterols designated as sterol A and sterol B were recrystalised from alcohol to get pure sterols.

Animals

Albino rats of both the sex weighing 150-200 g were used in the experimental study (CPCSEA Reg No. 221). They were maintained at standard laboratory conditions like temperature, relative humidity and dark/light cycle, they were fed with standard diet (Hindustan Liver India) and water ad libitum.

Induction of diabetes

Diabetes was induced in rats by intraperitoneal administration of Alloxan monohydrate (150 mg/kg b. w,) in normal saline. After 72 hrs, rats with hyperglycemia (more than 150 mg/dl) were selected and used for anti-diabetic evaluation. Blood glucose was measured by glucometer (Medicine optium).

Experimental Design

The rats were divided into 8 groups, each group consisted six rats. The *Butea monosperma* flower extracts suspended in tween 80 was administered orally by gastric intubation, after an over night fast. Normal control untreated rats (Group 1), diabetic control untreated rats (Group 2) were fed with only distilled water, Group 3 – Group 7 were treated with various extracts orally at a dose of 200 mg/kg b. w. daily for seven days and Group 8 animals were treated with standard drug Glibenclamide 10 mg/kg b. w, orally. Blood samples were collected from tail vein puncturing for measurement of blood glucose level for 1st day and at 7th day (for prolonged treatment) for isolated sterol A and B, blood glucose level was monitored at initial 1, 3, 5, 7 hrs of administration of 100 mg/kg b. w, orally (for acute study).

Statistical Analysis

The data was analysed by one way ANOVA followed by Dunnet's tests [9] at a level of significance $P < 0.0001$.

Result and Discussion

As per the preliminary phytochemical screening major phytoconstituents like sterols were present in petroleum ether (40^0 – 60^0) and in chloroform extract, flavanoids in ethyl acetate and alcoholic extract, but carbohydrates, proteins, aminoacids and phenolic compound were present only in aqueous extracts.

As per the result obtained from (Table 1) Alloxan induced diabetic rats petroleum ether, ethyl acetate and alcoholic extracts showed highly significant activity ($P < 0.0001$) and aqueous extract showed significant activity ($P < 0.01$) in prolonged treatment which were comparable to Glibenclamide but chloroform extract did not showed significant activity.

As per anti-diabetic study (Table 2) sterol A and sterol B were potent anti-diabetic agents.

From overall results petroleum ether extract containing sterols as a major chemical constituents having potent anti-diabetic activity. Ethylacetate and alcoholic extracts showed significant activity and this may be due to flavanoids.

Flavanoids are good antioxidants [10] and this will enhance the anti-diabetic activity.

Acknowledgement

The authors wish to thank Prof. A. V. Kulkarni, Department of Botany, G. S. Science Institute, Belgaum Karnataka, India for identifying the flower.

TABLE NO. 1 - EFFECT OF ISOLATED STEROLS OF *BUTEA MONOSPERMA* FLOWERS ON BLOOD GLUCOSE OF ALLOXAN DIABETIC ALBINO RATS AFTER PROLONGED TREATMENT

Groups (n)	Dose	Blood Glucose level mg/100dl (Mean±SEM)	
		Initial	7th day
Normal control (6)	2ml saline	106.16±3.27	106.50±3.73
Diabetic control (6)	2ml saline	282.66±18.50	282.66±19.37
Petroleum ether extract (6)	200mg/kg.b.w.	474.66±11.79	141.00±13.77***
Chloroform extract (6)	200mg/kg.b.w.	455.33±15.74	445.83±16.29
Ethyl acetate extract (6)	200mg/kg.b.w.	476.50±9.60	103.66±2.47***
Alcohol extract (6)	200mg/kg.b.w.	418.33±13.50	213.66±18.24***
Aqueous extract (6)	200mg/kg.b.w.	457.50±21.45	371.66±26.72*
Glibenclamide (6)	10mg/kg.b.w.	456.83±20.31	116.50±6.29***

n=6, *p<0.01, ***P<0.0001 v/s Control. SEM: Standard Error Mean, n=Number of animals

GRAPH – I

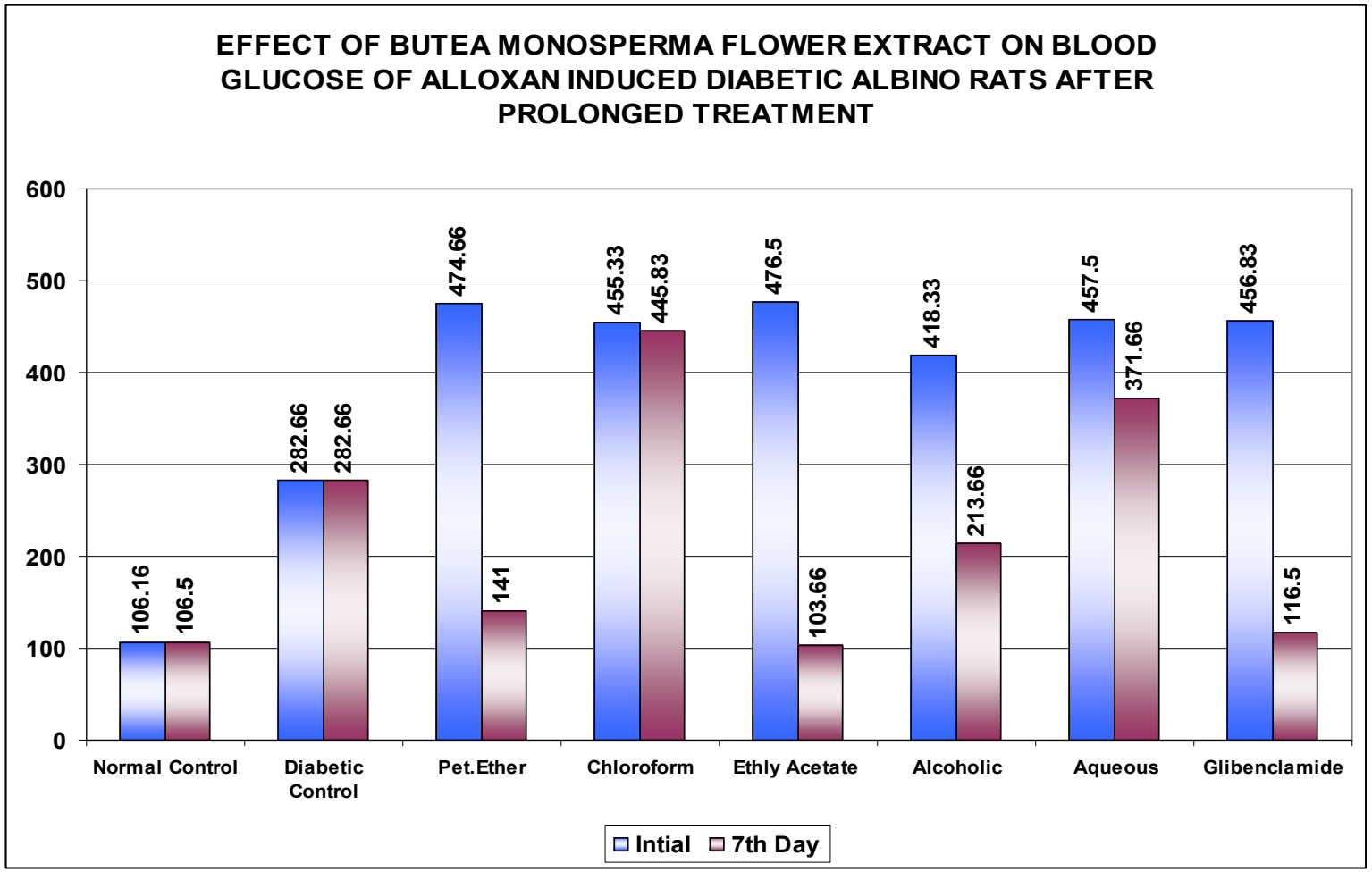

EFFECT OF BUTEA MONOSPERMA FLOWER EXTRACT ON BLOOD GLUCOSE OF ALLOXAN INDUCED DIABETIC ALBINO RATS AFTER PROLONGED TREATMENT
600
500
400
300
200
100
0
106.16
106.5
282.66
282.66
474.66
141
455.33
445.83
476.5
103.66
418.33
213.66
457.5
371.66
456.83
116.5
Normal Control
Diabetic Control
Pet.Ether
Chloroform
Ethly Acetate
Alcoholic
Aqueous
Glibenclamide
Intial
7th Day

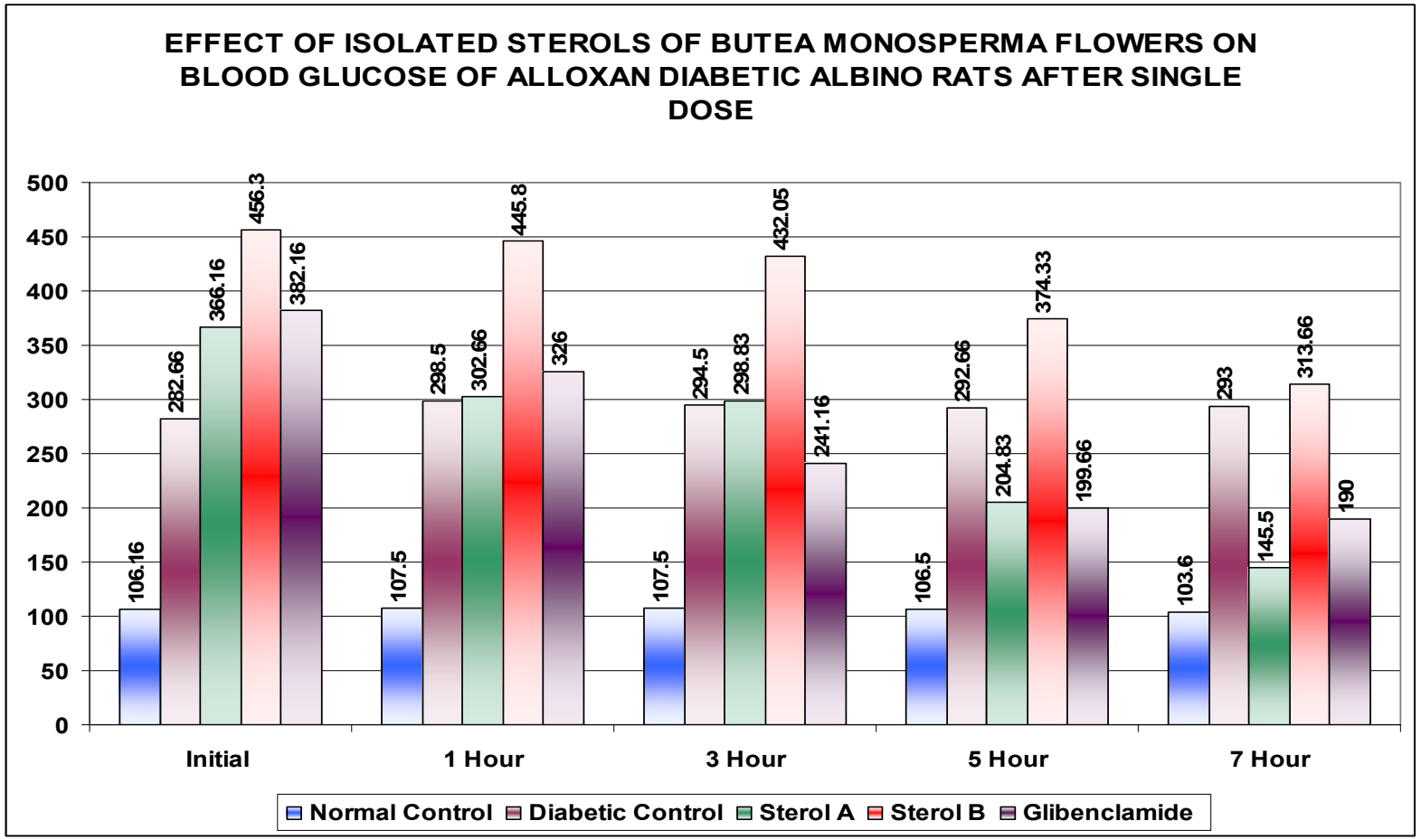
TABLE NO. 2 - EFFECT OF ISOLATED STEROLS OF *BUTEA MONOSPERMA* FLOWERS ON BLOOD GLUCOSE OF ALLOXAN DIABETIC ALBINO RATS AFTER SINGLE DOSE

Groups (n)	Dose	Blood Glucose level mg/100dl (Mean±SEM)				
		Initial	1hour	3hour	5hour	7hour
Normal control (6)	2ml saline	106.16±3.27	107.50±4.42	107.0±4.42	106.5±2.66	103.60 ±3.27
Diabetic control (6)	2ml saline	282.66 ±18.5	298.50±6.04	294.50±6.9	292.66±6.06	293±5.59
Sterol A	100mg/kg.b.w.	366.16±28.69	302.66±6.60	298.83±6.10	204.83±10.57*	145.50±5.94*
Sterol B	100mg/kg.b.w.	456.30±16.22	445.80±9.21	432.5±8.67	374.33±13.57*	313.66±20.43*
Glibenclamide (6)	10mg/kg.b.w.	382.16±27.0	362.0±7.81	241.16±4.88	199.66±4.6*	190±4.42*

n=6, *p<0.01 v/s Control.

SEM: Standard Error Mean, n=Number of animals

GRAPH – II


EFFECT OF ISOLATED STEROLS OF BUTEA MONOSPERMA FLOWERS ON BLOOD GLUCOSE OF ALLOXAN DIABETIC ALBINO RATS AFTER SINGLE DOSE
500
450
400
350
300
250
200
150
100
50
0
106.16
282.66
366.16
456.3
382.16
107.5
298.5
302.66
445.8
326
107.5
294.5
298.83
432.05
241.16
106.5
292.66
204.83
374.33
199.66
103.6
293
145.5
313.66
190
Initial
1 Hour
3 Hour
5 Hour
7 Hour
Normal Control
Diabetic Control
Sterol A
Sterol B
Glibenclamide

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