

## STUDY OF SOME MEDICINAL PLANTS FOR ANTIDIABETIC ACTIVITY IN ALLOXAN INDUCED DIABETES

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### Summary

The antidiabetic effects of hydroalcoholic extracts of *Acacia Arabica*, *Benincasa hispida*, *Tinispora cordifolia* and *Ocimum sanctum* were investigated in diabetic rats. The Alloxan monohydrate was used to induce the diabetes in normal rats. The tolbutamide 80 mg/kg p.o. was used the standard antidiabetic throughout the study. Our results indicated that 250 and 500 mg/kg b.w. of all hydroalcoholic test extracts reversed the altered glucose, cholesterol, triglycerides, LDL and HDL levels in diabetic rats significantly and in dose dependent manner. Hence, the study reveals the usefulness and beneficial value of herbal drugs in the treatment of diabetes.

**Key Words:** Diabetes, alloxan induced diabetes, tolbutamide

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### Introduction

The number of people with diabetes is increasing day by day, the main cause of this problem is aging, urbanisation and increasing prevalence of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future it is important to have rational planning and allocation of resources towards treatment and prevention of this disease<sup>1,2</sup>. Diabetes mellitus is a chronic metabolic disorder caused by an absolute or relative lack of insulin and or reduced insulin activity which results in hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism<sup>3,4</sup>.

In the present study, the hydroalcoholic extracts of *Acacia Arabica*, *Benincasa hispida*, *Tinispora cordifolia* and *Ocimum sanctum* were evaluated for antidiabetic activity. *Acacia arabica* (Linn.) Willd. Ex Del. Ssp. Indica (Benth.) Brenan belongs to the family Mimosaceae. It is a moderate sized tree distributed throughout India. *Acacia arabica* is generally used for various diseases and disorders. *Acacia arabica* showed significant anti diabetic and antioxidant activity<sup>5,6</sup>.

*Benincasa hispida* (Thunb.) Cong. *Benincasa hispida* belongs to the family Cucurbitaceae and is cultivated throughout the India. Its fruits and seeds are medicinally used as laxative, diuretic, tonic, aphrodisiac and periodic. They are also useful in asthma, cough, diabetes, haemoptysis and hemorrhages from internal organs, epilepsy, fever and vitiated conditions of pitta. *Benincasa hispida* showed significant reduction of glucose<sup>6,7,8</sup>.

*Tinispora cordifolia* (Wild.) Miers ex Hook. F. & Thoms. *Tinispora cordifolia* belongs to family Menispermaceae distributed throughout the India. It is medicinally used for various diseases and disorders. *Tinispora cordifolia* showed significant reduction in sugar, showed anti-oxidant activity<sup>9,10,11,12</sup>.

*Ocimum sanctum* Linn. (*Ocimum sanctum* linn) belongs to Lilacea family and distributed throughout India. *Ocimum sanctum* showed significant reduction of sugar<sup>13,14,15</sup>.

## **Materials and methods**

### ***Chemicals and Drugs***

Alloxan monohydrate was purchased from (Sigma Chemicals St. Louis, U.S.A.), Chem. Kit for cholesterol estimation (Erba Diagnostics Mannheim), Chem. Kit for Triglycerides estimation (Erba Diagnostics Mannheim), Chem. Kit for Glucose Estimation (Erba Diagnostics Mannheim), Chem. Kit for HDL and Chem.kit for LDL (Erba Diagnostics Mannheim)

### ***Plant Material***

The bark of *Acacia Arabica*, fruit of *Benincasa hispida*, stem of *Tinispora cordifolia* and arial part of *Ocimum sanctum* was collected from Pune district (India) in August 2008. Samples were authenticated by Head of The Department, Department of Botany, Sharadabai Pawar College Malegaon(bk) Tal- Baramati Dist- Pune.. The voucher specimens ( AA-08, BH-08, TC-08 and OS-08 are deposited in herbarium of Sharadabai Pawar College Malegaon(bk) Tal- Baramati Dist- Pune.

### ***Preparation of Extract:***

#### ***Hydro alcoholic extracts (AAH, BHH, TCH and OSH)***

Each of the dried samples (100gm) of the plant was extracted by Soxhlet extraction with water and 95% ethanol (6:4 v/v) at controlled temperature.

### ***Experimental animals***

The male albino rats of Wistar strain 150 – 200 g and albino mice 20 – 30 g was used throughout the experimentation. Rats and mice are procured from SVPM's College of Pharmacy, Malegaon (bk), Tal- Baramati Dist- Pune 413115. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry condition as follows

Room temperature:  $27 \pm 3^{\circ}\text{C}$

Relative humidity:  $65 \pm 10\%$

12 hrs. Light/dark cycle

All the animals were fed with rodent pellet diet (Gold mohr, Lipton India Ltd.,) and water was allowed ad-libitum under strict hygienic condition. Ethical clearance for performing experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC).

### ***Toxicity Studies***

An acute toxicity of hydro alcoholic and chloroform extracts were carried out in female albino mice (20 – 30 g), those maintained under standard conditions. The animals were fasted over night prior to the experiment. Fixed dose (OECD Guideline No. 420) method of CPCSEA was adopted for toxicity studies (Mrs. Prema Veeraraghavan. Expert consultant, CPCSEA, OECD guide line No. 420; Oct 2000)

***Preparation of diabetic rats***

The experiments were carried out with male albino rats of Wistar strain weighing 140-180 g. Alloxan monohydrate dissolved sterile normal saline at dose of 150 mg/kg b.w., intraperitoneally. After a fortnight, rats with marked hyperglycemia were selected and used for the study.

***Experimental Design*<sup>16,17,18,19</sup>**

In the experiment a total 55 rats (50 diabetic rats, 5 normal rats) were used. Diabetes was induced in rats 2 week before starting the treatment. The rats were divided into eleven groups as follows after the induction of alloxan diabetes and each group comprised of 5 rats. Group I (untreated normal rats), group II (untreated diabetic rats), group III (diabetic rats receiving tolbutamide orally at 80 mg/kg in distilled water), group IV to XI (diabetic rat treated with hydroalcoholic test extract at dose 250 and 500 mg/kg b.w. of all plants). Tolbutamide 80 mg/kg p.o was used the standard antidiabetic throughout the experimentation.

The animals were carefully monitored every day. Animals described as fasted were deprived of food for at least 12 h but allowed free access to drinking water. Blood samples were drawn at weekly intervals till the end of study. Fasting blood glucose measurement was done on day 0, 7, 14 of the study. On day 14, under mild ether anesthesia blood was collected by cardiac puncture and processed for estimation of serum glucose and serum lipid profile.

***Biochemical estimation***

Fasting serum was collected for estimation of biochemical parameters, such as serum glucose (GOD-POD method), cholesterol (CHOD-PAP method), triglycerides (GPO-Triender method), HDL cholesterol and serum LDL.

***Statistics***

All values were expressed as Mean  $\pm$  ESM. The differences were compared using one way analysis of variance (ANOVA) followed by Turkey Kramer. P values < 0.05 were considered as significant.

**Results**

The blood glucose was increased significantly in untreated alloxan- diabetic rats as compared to normal rats. Administration of different hydroalcoholic extract of different plant part and tolbutamide decrease in glucose levels in diabetic treated groups. Serum cholesterol, triglycerides, HDL, LDL levels also reversed with the treatment of test extracts and standard. The results of glucose estimations are showed in table 1 and the results of cholesterol, triglycerides, LDL, HDL are tabulated in table 2.

**Discussion**

Alloxan has been observed to cause a massive reduction of the  $\beta$  cells of the islets of Langerhans and induce hyperglycemia<sup>20</sup>. Diabetes mellitus is a metabolic disorder, showing significant impact on lipid metabolism with alterations in blood lipids and lipoproteins profile. Absence or deficiency of insulin alters the entire metabolism in the body including the lipid metabolism. The abnormal high levels of blood lipids in diabetes are mainly due to increase in mobilization of free fatty acids from the peripheral depots (increased lipolysis) as hormone sensitive lipase is not inhibited in diabetes due to insulin lack. The marked hyperlipemia, which characterizes the diabetic state, may therefore be considered as consequences of the unhibited actions of lipolytic hormones on the fat depots<sup>21</sup>.

Table 1: Effect of treatment of different extracts AAH, BHH, TCH, OSH at the doses 250 and 500 mg/kg b.w. for 2 weeks on serum glucose concentration in diabetic rats.

Group/ Treatment	Average serum glucose (mg/dl)		
	Day 0	Day 7	Day 14
Untreated Normal	85.25±6.23	90.35±5.42	96.48±7.59
Untreated Diabetic	336.21±3.26	358.57±4.36	362.32±8.26
Diabetic+ Tolbutamide	297.26±4.56	186.60±5.26 <sup>***</sup>	105.37±4.87 <sup>***</sup>
Diabetic + BHH 250 mg/kg	325.81±2.69	328.63±3.26 <sup>**</sup>	285.29±6.19 <sup>***</sup>
Diabetic + BHH 500 mg/kg	315.27±4.12 <sup>***</sup>	275.46±2.69 <sup>***</sup>	120.20±4.68 <sup>***</sup>
Diabetic + AAH 250 mg/kg	330.31±3.62	328.60±6.18 <sup>**</sup>	264.54±8.24 <sup>***</sup>
Diabetic + AAH 500 mg/kg	322.12±4.12	260.53±5.49 <sup>***</sup>	117.36±6.13 <sup>***</sup>
Diabetic + TCH 250mg/kg	335.71±4.18	326.33±6.89 <sup>**</sup>	276.46±4.59 <sup>***</sup>
Diabetic + TCH 500mg/kg	298.92±5.13 <sup>***</sup>	250.63±4.51 <sup>***</sup>	112.76±3.91 <sup>***</sup>
Diabetic + OSH 250mg/kg	332.81±6.18	332.73±5.68 <sup>*</sup>	271.89±6.25 <sup>***</sup>
Diabetic + OSH 500mg/kg	312.39 ±3.59 <sup>*</sup>	203.68±3.26 <sup>***</sup>	106.56±6.32 <sup>***</sup>

(BHH- Hydroalcoholic extract of Benincasa hispida AAH- Hydroalcoholic extract of Acacia arebica, TCH- Hydroalcoholic extract of Tinospora cordifolia, OSH- Hydroalcoholic extract of Ocimum sanctum)

Values are expressed as mean ±SEM; (n = 5)

\*p < 0.05, \*\*\*p < 0.001 compared with untreated diabetic rats.

Table 2: Effect of treatment of different extracts AAH, BHH, TCH and OSH at the doses 250 and 500 mg/kg b.w. for 2 weeks on serum cholesterol, triglycerides, HDL and LDL (mg/dl) in diabetic rats

Group/Treatment	Average serum lipid profile (mg/dl)			
	Total Cholesterol	Triglycerides	HDL Cholesterol	LDL Cholesterol
Untreated Normal	64.28 ± 3.51	79.64 ± 3.12	48.60 ± 3.72	48.53 ± 4.36
Untreated diabetic	95.28 ± 4.01	143.88 ± 3.52	26.14 ± 5.61	108.23 ± 7.59
Diabetic+ Tolbutamide	58.78 ± 3.61***	81.10 ± 4.00***	41.86 ± 5.94**	55.34 ± 5.46**
Diabetic + BHH 250 mg/kg	68.88 ± 3.84*	110.44 ± 4.12*	31.26 ± 6.34	91.37 ± 4.64
Diabetic + BHH 500 mg/kg	61.29 ± 5.65**	92.59 ± 5.61**	38.56 ± 4.84**	67.46 ± 3.45*
Diabetic + AAH 250 mg/kg	71.56 ± 4.85	102.69 ± 6.18	32.46 ± 7.53	89.97 ± 5.41
Diabetic + AAH 500 mg/kg	59.29 ± 5.85**	85.29 ± 5.43**	36.48 ± 5.13*	73.83 ± 3.61**
Diabetic + TCH 250 mg/kg	73.56 ± 6.35	98.84 ± 4.12*	32.54 ± 3.47	87.54 ± 3.26
Diabetic + TCH 500 mg/kg	64.59 ± 5.48**	82.34 ± 5.48**	40.19 ± 6.79**	59.36 ± 4.16**
Diabetic + OSH 250 mg/kg	78.57 ± 7.35	85.44 ± 4.12**	30.46 ± 4.69	85.56 ± 5.18
Diabetic + OSH 500 mg/kg	62.85 ± 6.62**	86.13 ± 5.31***	41.68 ± 6.15**	58.23 ± 6.48**

(BHH- Hydroalcoholic extract of Benincasa hispida AAH- Hydroalcoholic extract of Acacia arabica, TCH- Hydroalcoholic extract of Tinospora cordifolia, OSH- Hydroalcoholic extract of Ocimum sanctum)

Values are expressed as mean ± SEM; (n = 5)

\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 compared with untreated diabetic rats.

In the present study, the serum glucose, cholesterol, triglycerides, and LDL in the diabetic animals were elevated to high levels during the study period, which is in well agreement with earlier reports<sup>22,23</sup>. Treatment of test drugs for 2 weeks in diabetic rats showed a significant reduction in all these parameters compared to untreated diabetic animals. High density lipoprotein (HDL) cholesterol is produced in both liver and intestine.

HDL constituents are also derived from chylomicron and VLDL catabolism<sup>24</sup>: HDL serves as an acceptor of lipids, especially free cholesterol from various extra hepatic cells to the liver for the ultimate excretion in the bile<sup>25</sup>.

Phytochemical analysis of used plant extracts shows the presence of alkaloids, flavonoids, saponins and steroids. Some plants exhibit properties similar to the well known sulfonylurea drugs like tolbutamide; they reduce blood glucose in normoglycemic animals<sup>26,27</sup>. Flavonoids, sterol/triglycerides, alkaloids and and phenolics are known to be bioactive antidiabetic principles.

### **Conclusion**

The study concludes that, hydroalcoholic extracts of *Acacia Arabica*, *Benincasa hispida*, *Tinispora cordifolia* and *Ocimum sanctum* ameliorate the glucose level and metabolic derangements in lipid caused by alloxan induced diabetes in rats. Efficacy of this activity is appreciably good when compared to standard drug tolbutamide.

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