

Research Article**Phytochemical and Pharmacological Investigation of *Hyptis suaveolens* L. in Experimental Models***Dharmendra Singh¹, Anil Kumar Middha²¹Research Scholar, OPJS, University, Churu, Rajasthan, India²Associate Professor, OPJS, University, Churu, Rajasthan, India

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ABSTRACT

Hyptissuaveolens L. Poit is a plat belonging to family Lamiaceae, or the Mint family. The original family name is Labiatae, while this is still considered a suitable alternate name nearly all botanists now use the name "Lamiaceae" in referring to this family. It is also called *Ballotasuaveolens* Linn, (Kirtikar and Basu, 1999). The word *diabetes* is Greek for a draw off, referring to the ejection of a more quantity of urine; and *mellitus* is Latin used for sugar. Consequently diabetes mellitus means the passage of huge amounts of sweet urine. The plant materials (1 kg) were initially defatted with petroleum ether and then extracted with alcohol and water using a Soxhlet apparatus. The yield of the plant extracts ethanol (95%) and aqueous measured about 20 g each after evaporating the solvent using water bath. The standard extracts obtained from *Hyptissuaveolens*L. Were then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening. Diabetes was induced by intra-peritoneal injection of Alloxan monohydrate (150 mg/kg b.w.) dissolved in the in normal saline (Viana et al, 2004). Blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. Animals were considered diabetic when the blood glucose level was raised beyond 200 mg/100 ml of blood. This condition was observed at the end of 72 h after alloxanisation.

Keywords: *Hyptis suaveolens* L, Lamiaceae, Alloxan, diabetic, *mellitus***INTRODUCTION:**

The generic *Hyptis* has been derived from Greek word *Hyptios* laid back or resupinate, referring to the limb of the corolla, which is turned on its back. It is a large genus of about 400 species and is native of warm tropical America.

Table 1:

Vernacular names	
Hindi	Ganga tulsi, ban-tulsi, bilayatitulsi
Bengali	Bilaiti-tulsi
Oriya	Ganga-tulsi, purodo
Telguu	Sima-tulsi, mahavira
French	Gros baume, hyptis à odeur
Spanish	Chao, hierba de lasmuelas, hortela do campo
Portuguese	Joa-de-capote
Guarani	Kamamb

Botanical description

Strong-scented herb to 3 m tall with quadrate hairy, erect, branched stems. When crushed, the plant gives off a characteristic minty smell. The broad leaves are in opposite pairs up the stem, with small mauve flowers in clusters in the upper leaf axils. Leaves, ovate, acute, 3-5 cm long and 2-4 cm wide, the margins serrulate, lower surface densely hairy; petioles up to 3 cm long. Inflorescence, axillary, 3-to 4-flowered clusters, flowers in small cymes, along branch end with reduced leaves. Calyx 5 mm long, in flower, 10 mm long in fruit, corolla 2-lipped, purplish blue. Fruits ribbed enclosed by the calyx. Nutlets about 1.2-1.5 mm long slightly notched at the end." The persistent spiny calyx enclosing the seeds assists with their dispersal by adhering to clothing, fur and wool. The plant gives flowers and fruits in autumn and winter seasons.



Figure 1: Plant of *HyptissuaveolensPoiit* (L)

EXPRIMENTAL WORK

Preparation of Interventions

The measured quantity of extracts and fractions of *HyptissuaveolensPoiit* and the standard drug glibenclamide (5 mg/kg) was suspended in 25% Tween-20 in distilled water. The solvent, test samples and standard drugs were administered by oral route based on dose and corresponding weight of the animals. For oral administration of test, standard as well as Solvent Feeding needle no 21 was used.

Maintenance of animals and Exposure Conditions

Earlier to the experiments, the selected animals were housed in acrylic cages in standard environmental conditions (conditions (temp: 20–25 °C; relative humidity: 45-55 % under 12 h light/dark cycle), fed with standard rodent diet for 1 week in order to adapt to the laboratory conditions and water *ad libitum*. They were fasted overnight (12 h) before experiments, but were allowed free access to water. Six animals were used for each group of study. All the experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Ethical Committee (IAEC No. 1171/C/08/CPCSEA).

Acute oral toxicity study of extracts

The method of Ghosh, 2005 was followed. Six groups for alcoholic, aqueous and hydro-alcoholic extracts of aerial parts *HyptissuaveolensPoiit* ten mice each, of mixed sex fasted overnight were reserved under laboratory conditions and allowed

free access to water. The test extracts at increasing concentrations dissolved in distilled water were administered orally via a gastric catheter. After administration of test samples, the animals were observed significantly for the first 4 h for any behavioral changes, followed by occasional observation for 6h and finally mortality was recorded after 72 hours.

Blood glucose level determination

Fasting blood glucose concentration was determined using a Glucometer (Optium), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns (Aslan et al, 2007a, Aslan et al, 2007b)

Antihyperglycemic activity of extracts in glucose-loaded animals (oral glucose tolerance test)

The oral glucose tolerance test (OGTT) measures the body's ability to use main source of energy i.e. glucose. OGTT is to simplify and facilitate the diagnosis of diabetes (Luzi, 1998). This method is frequently referred to as physiological induction of diabetes mellitus because the blood glucose level of the animal is fleetingly increased with no damage to the pancreas. An oral glucose tolerance test (OGTT) was performed on diabetic rats by feeding glucose (5 g/kg) per os. Animals were deprived of food 18 h before and during the experiment, but were allowed free access to water. They were divided into 7 groups of 6 rats each. Group I served as normal control, Group II served as solvent control and received only vehicle (Tween + water - 2 ml/kg b.w.) through the oral route. Group III received glibenclamide (5 mg/kg b.w.). Groups IV to VII received the alcohol and aqueous extracts of *HyptissuaveolensL.* at a dose of 200 and 400 mg/kg b.w., respectively, through oral route. The blood glucose level was determined before drug and glucose administration (-1 and 0 h, respectively) and subsequently at 0.5, 1, 2 and 3h after.

RESULT AND DISCUSSION

Results of acute oral toxicity studies of different extracts of *H. suaveolens*

Acute toxicity studies conducted revealed that the administration of graded doses of both the crude aqueous and ethanol extracts (up to a dose of 5000 mg/kg) *H. suaveolens* did not produce

significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and appearance of the animals. No death was observed up to the dose of 5 g/kg body weight. The mice were physically active. These effects were observed during the experimental period (72 hrs). The result showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD₅₀) could be greater than 5 g/kg body

weight in mice. Based on these results 1/10th of the maximum safest dose was taken for further pharmacological screening. So the doses selected for further study were 250 mg/kg b.w. and 500 mg/kg b.w. for *H. suaveolens*. Hydro-alcoholic extract also did not show any toxic effect up to 5 g/kg, so average lethal dose (LD₅₀) for hydro-alcoholic extract was 250 mg/kg b.w. and 500 mg/kg b.w. Results are depicted in table 6.12.

Table 1: Acute oral toxicity study of different extracts of *H. suaveolens*

Group	Dose (mg/kg)	Route	Death/Total in Ethanolic extract	Death %	Death/Total in Aqueous extract	Death %
I	500	Oral	00/10	0	00/10	0
II	1000	Oral	00/10	0	00/10	0
III	1500	Oral	00/10	0	00/10	0
IV	2000	Oral	00/10	0	00/10	0
V	3000	Oral	00/10	0	00/10	0
VI	5000	Oral	00/10	0	00/10	0

Result: Mortalities after 72hrs were recorded as shown in the Table.

Table: 2 Effect of ethanolic and aqueous extracts of *H. suaveolens* oral glucose tolerance in normal rats

Gr.	Treatment and dose	Blood glucose concentration (mg/dl)					% decrease at end of 3hr
		0 min	30 min	60 min	120 min	180 min	
I	Normal Control	83.75 ± 0.47	86.50 ± 0.98	88.50 ± 0.64	83.50 ± 0.64	86.50 ± 0.61	
II	Solvent control	90.50 ± 0.64	135.52 ± 0.64**	118.83 ± 0.85**	98.50 ± 0.64**	91.50 ± 0.44**	32.48
III	Glibenclamide (5mg/kg)	89.43 ± 0.40	95.50 ± 1.04**	81.53 ± 0.91**	72.50 ± 0.64**	62.51 ± 0.72**	34.54
IV	Eth. Ext. (250mg/kg)	83.62 ± 0.40	98.25 ± 0.85**	97.61 ± 0.91**	93.50 ± 0.64**	89.13 ± 0.34	9.28
V	Eth. Ext. (500mg/kg)	87.50 ± 0.64	107.31 ± 1.37**	102.32 ± 1.10**	94.50 ± 0.64*	91.50 ± 0.64	15.73
VI	Aq. Ext. (250mg/kg)	91.50 ± 0.64	116.84 ± 1.10**	109.83 ± 0.85**	102.65 ± 0.91*	92.50 ± 0.54**	20.83
VII	Aq. Ext. (500mg/kg)	82.97 ± 0.91	128.36 ± 0.85**	113.36 ± 1.10	101.51 ± 0.64**	94.51 ± 0.65**	25.89

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test (t-value denotes statistical significance at *p<0.05, **p<0.01 respectively, in comparison to diabetic control group).

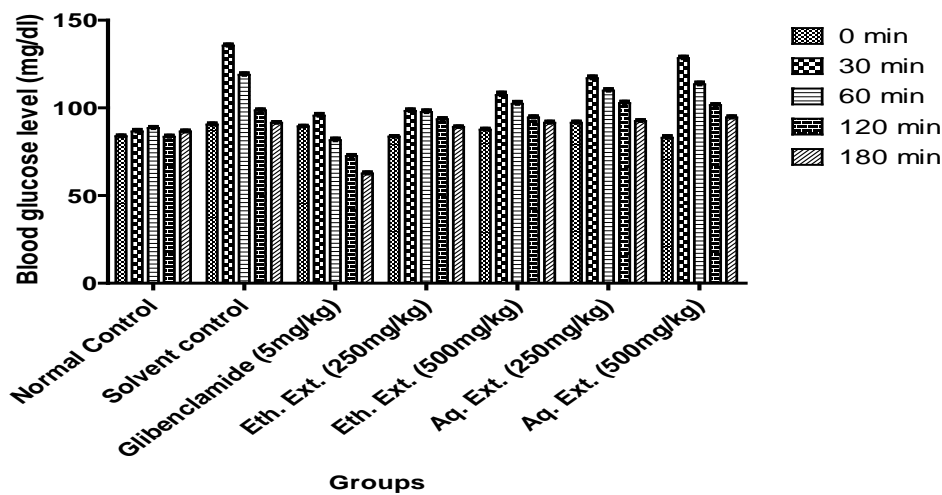


Figure 2: Effect of ethanolic and aqueous extracts of *H. suaveolens* oral glucose tolerance in normal rats.

Effect on blood glucose level of the extracts in alloxan induce hyper glyceemic rats (Acute and sub-acute models)

Acute model (In single dose treated alloxan induced hyperglycemic rats)

The effects of ethanol and aqueous extracts of aerial parts of *H. suaveolens* on fasting blood glucose levels on single dose treated alloxan induced diabetic rats are presented in table 6.15. The ethanol and aqueous extracts at 250 mg/kg dose level registered 134.36, 131.72 mg/dl at the end of 10 h of the study, while it was 126.59, 119.34 mg/dl with dose level of 500 mg/kg. However at the same time the standard drug glibenclamide at 5mg/kg showed 92.59 mg/dl of

BGL. A dose dependent effect of the test extracts was observed. The ethanol and aqueous extracts of *H. suaveolens* both dose levels (250 and 500 mg/kg) showed a persistent decrease in blood glucose level till the end of 10 hr., with maximal decrease noted in aqueous extract at 500 mg/kg dose, reaching 66.72% ($p < 0.01$), while the standard drug glibenclamide showed 70.67% decrease. The statistical significance of one way ANOVA showed significant reduction of BGL @ $p < 0.05$ to 0.01 starting from 1 hr up to the end of 10 hr within the groups. The potency order of the test extracts towards the falling of BGL is aqueous extract followed by ethanolic extract.

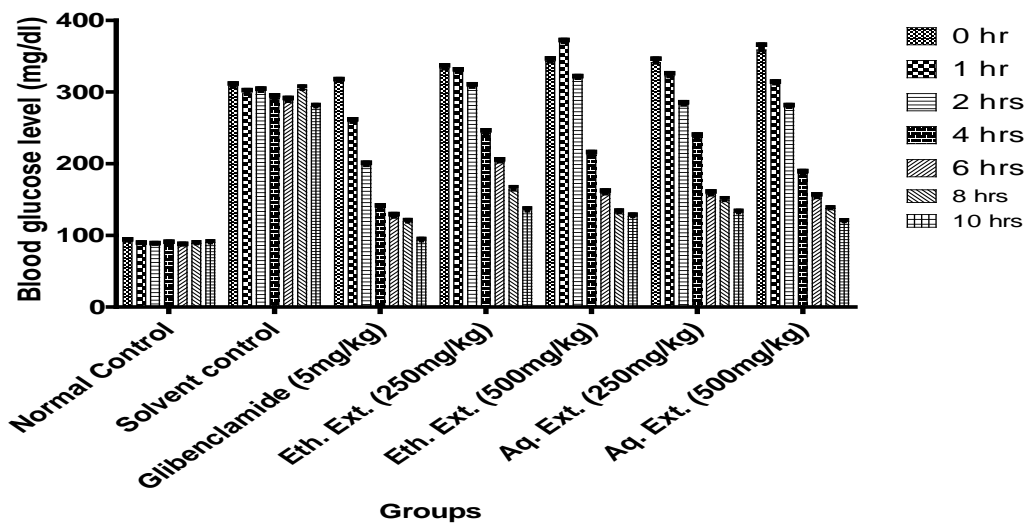


Figure 3: Effect of ethanolic and aqueous extracts of *H. suaveolens* on blood glucose level in single dose treated alloxan induced hyperglycemic rats

CONCLUSION

The present investigations concluded that the ethanolic and aqueous extracts of aerial parts of *H. suaveolens* endowed with potential antidiabetic activity which could be attributed by their possible multiple effects on both pancreatic and extra-pancreatic site by influencing either the metabolism and/or absorption of glucose, which in turn also influence the lipid metabolism. On the other hand, hydro-alcoholic extract, chloroform and aqueous fraction of *H. suaveolens* also possess potential hypoglycaemic and antihyperglycaemic activity. Conversely the extracts and fractions of both plants exert very good potentials to scavenge toxic free radicals along with the inhibition of the liver lipid peroxidation products and activation of the enzymatic antioxidant defense mechanism in diabetic rats that might be due to the presence of high levels of sterols, phenolics, alkaloids and flavonoids, which may be responsible for the supporting properties of the extracts and fractions for their hypoglycaemic and antidiabetic activity.

REFERENCES

1. Ziegler, H.L., et al., 2002. Possible artefacts in the in vitro determination of antimalarial activity of natural products that incorporate into lipid bilayer: Apparent antiplasmodial activity of dehydroabietinol, a constituent of *Hyptissuaveolens*. *PlantaMedica* 68(6), 547-549.
2. Taiwo, A., Ekundayo, O., Adeniyi, B., 1999. Antimicrobial activity of the essential oil of *Hyptissuaveolens* leaves. *Fitoterapia* 70(4), 440-442.
3. Tanaka, K., Nishizon, O.S., Makino, N., Tamaru, S., Terai, O., Ikeda, I., 2008. Hypoglycemic Activity of *Eriobotrya japonica* Seeds in Type 2 Diabetic Rats and Mice. *Bioscience, Biotechnology, and Biochemistry* 72 (3), 686-693.
4. Zhang, H., Zdolsek, J.M., Brunk, U.T., 1992. Alloxan cytotoxicity involves lysosomal damage. *Acta Pathologica Microbiologica EtImmunologicaScandinavica* 100, 309-316.
5. Zhang, L.H., Xiao, P.G., 1993. Recent advances in Studies of Antihyperlipaemic and Antihyperglycemic compounds from Chinese traditional and herbal medicines. *Phytotherapy Research* 7, 217-226.
6. Zhang, X., and Tan, B.K.H., 2002. Antihyperglycemic and antioxidant properties of *Andrographispaniculata* in normal and diabetic rats. *Clinical and Experimental Pharmacology and Physiology* 27, 358-63.
7. Zhang, X.F., Tan, B.K., 2000b. Anti-hyperglycaemic and antioxidant properties of *Andrographispaniculata* in normal and diabetic rats. *Clinical and Experimental Pharmacology and Physiology* 27, 358-363.
8. Wagner, H., Bladt, S., 1996. *Plant Drug Analysis: A Thin Layer Chromatography Atlas*. Second edition. Springer Verlag Berlin Heidelberg New York Tokyo, 384.
9. Walter, R.M., Uriu-Hare, J.Y., Lewis, O.K., 1991. Copper, zinc, manganese, and magnesium status and complications of diabetes mellitus. *Diabetes Care* 14, 1050-1056.
10. Walters, W.M., Namchuk, M., 2003. A guide to drug discovery: Designing screens: how to make your hits a hit. *Nature Reviews Drug Discovery* 2, 259-266.