EVALUTION OF ANTIDIABETIC ACTIVITY OF O*LEA-EUROPEA* IN WISTAR ALBINO RATS

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ABSTRACT: In the present study attempts were made to study anti-diabetic activity of the leaves of *Olea europea* belonging to the family oleaceae. Ethanolic and Petroleum ether extracts of *Olea europea* use used and compared with Metformin as standard drug (500mg/kg). Wister strain of either sex was treated with Alloxan (150mg/kg) to induce diabetes. Glucose Oxidase/Perioxidase method was used for the determination of plasma glucose level. The ethanolic extract showed significant decrease in blood glucose level.

Keywords: *Olea europea*, leaves, Antidiabetic activity, Ethanolic extract.

INTRODUCTION

Olea europea belonging to the family oleaceae is a small evergreen tree, from 12 to 20 feet high, with hoary, rigid branches, and a grayish bark1 In many countries extract of *Olea europea* is used in the treatment, migraine, insomnia, diarrhea, dysentery, fever, pile and fistula[1,2].The phytochemical characteristics show the presence of tannins, steroids, alkaloids, triterpens and flavonoids. *Olea europea* is traditionally used in Indian system of medicine for the treatment of diabetes[1-5]. The aqueous extract of *Olea europea* has been reported for the treatment of hyperglycemic patients, but other parts of plants such as leaves or pods have not been studied. Hence it was thought worthwhile to screen different extracts of leaves of *Olea europea* for its anti-diabetic effects.

MATERIAL AND METHODS

Procurement of Plant Material

Plant material and drugs

The *Olea europea* were collected from local agricultural area around Pune city region. The plant specimen was authenticated. The drugs were purchase from different sources.

Extraction

Powdered leaves were charged into soxhlet apparatus and successive hot continuous extraction was carried out using solvents such as Petroleum ether (60-80C), and ethanol. The drug was extracted with each solvent. Each time before extraction with the next solvent, the powdered material was air dried. Each extract was concentrated by distilling the excess solvent to obtain the crude extract[6, 7, 8].

Preliminary Phytochemical Screening

The preliminary Phytochemical screening of both extracts were performed in order to find out the class or classes of the constituent presents in the extract[8].

Evaluation of Hypoglycemic Activity of Extracts

Normal healthy Wistar rats (200-250 g) of both sexes were used for present investigation. Animals were housed under standard environmental conditions at temperature (25±2°C), humidity (55±10%) and light and dark (12:12 h.). Rats were fed standard pellet diet (Godmother Brand, Lipton India Ltd., Mumbai.) and water *ad libitum*. Treatment GroupsThe animals were divided into 4 different groups and each group consists of 6 animals. Group-1: Control Group. Group-2: Standard Group- Diabetic rats were treated with Metformin orally (500 mg/kg/day). Group-3: Diabetic rats were treated with suspension of petroleum ether extract orally (500 mg/ kg/day). Group-4: Diabetic rats were treated with suspension of ethanolic extract orally (500 mg/kg/day).

Induction of *Diabetes*

The experimental induction of *diabetes* was carried out in rats by administration of alloxan (150mg/kg, i.p.). Each groups were subjected to overnight fasting then blood samples of each groups were collected from retro-orbital plexus by means of sterilized glass capillary tubes under light ether anesthesia, then the blood was cold centrifuged. Plasma was collected and used as sample. The glucose oxidized / peroxidase method was used for the determination of plasma glucose level in the rats[12-18].

RESULTS

Table 1: Hypoglycemic Activity of Different Extracts

Treatment	Dose mg/kg	Serum glucose level (mg/dl)			
		0h	1h	3h	5h
Control (saline)	----------	375 ±1.76	245 ±0.79	269.21±2.22	266.02±3.1
Standard (Metformin)	500	282.00±2.80	242.27±1.63	201.42*±4.21	159.11*±0.99
Pet. Ether Extract	500	201.34±3.41	199.72±4.72	195.67±2.91	187.12±3.55
Ethanolic Extract	500	282.18±5.59	244.68±6.75	200.53±3.02	180.58*±2.12

n = 6, values are presented as mean ± standard deviation
* P < 0.05 with respect to corresponding control.

Table 2: Hypoglycemic Activity of Different Extracts

Treatment	Dose mg/kg	Serum glucose level (mg/dl)			
		0h	1h	3h	5h
Control (saline)	---------	277.83±1.87	268.02±3.10	329.21±1.23	196.13±3.21
Standard (Metformin)	500	280.12±2.80	159.11±0.99	124.15*±2.63	105.21*±5.32
Pet. Ether Extract	500	272.67±2.91	257.12±3.55	239.43±0.36	168.96±3.26
Ethanolic Extract	500	254.36±5.22	170.58±2.12	144.32±1.03	106.21*±1.69

n = 6, values are presented as mean ± standard deviation
* P < 0.05 with respect to corresponding control.

Table 3: Thin Layer Chromatography of Ethanolic Extract

Sr. No.	Solvent System	No. of Spots in Iodine Vapours	Rf value
1		1	0.66
2	Chloroform: Methanol: n-hexane (5:2:2)	2	0.58
3		3	0.35

Screening of Hypoglycemic Activity of Extracts

Extracts obtained were subjected for evaluation of hypoglycemic activity in Alloxan-induced diabetic rats. Metformin (500mg/kg) was taken as standard. Ethanolic extract showed significant decrease in serum glucose level to 34.16% as compared to standard which was 44.50% at 5th hr after drug treatment. Petroleum ether extract did not show
significant decrease in serum glucose level. Results are given in Table-1. Subsequently, on 3rd and 7th day, the ethanolic extract further reduced serum glucose level with respect to corresponding control, but the pet. ether extract did not show any significant reduction in serum glucose level up to 7 days. The ethanolic extract reduced serum glucose level to
51.30% as compared to standard which was 63.11% on 7th day. The results are given in table 2.

Thin Layer Chromatography of Ethanolic Extract

As the ethanolic extract showed hypoglycemic activity, the extract was subjected to TLC. Various solvent systems were tried. The most suitable solvent system was Chloroform: Methanol: n-hexane (5:2:2). The spots were detected using iodine as a detecting reagent. The ethanolic extract showed 3 spots, which indicated the number of constituent present in ethanolic extract. The Rf values of these spots are given in table 3.

Column Chromatography of Ethanolic Extract

Three constituents were separated by column chromatography using gradient elution technique. The constituents separated were collected in fractions. The fractions were having similar pattern of TLC (same Rf value). They were mixed and solvents were evaporated.

DISCUSSION

In the present study attempts were made to study detail phytochemical and pharmacological, particularlyanti-diabetic activity of the leaves of *Olea europea* belonging to family oleaceae. On hot continuous extraction, the percentage extractive values were obtained are in petroleum ether (60-80^{0}C) 1.55 % w/w and in ethanol 3.00% w/w. The preliminary phytochemical screening of both extracts was performed in order to find out the class or classes of the constituent present in the extract. The various chemical constituents present in petroleum ether extract were sterols and triterpenoids, while in ethanolic extract were alkaloids, and flavonoids as active constituent [20, 21]. In the present study, *diabetes* was induced in rats by intraperitoneal injection of alloxan (150mg/k g b.w.). The petroleum ether and ethanolic extracts were fed to diabetic rats up to 7 days and serum glucose level was determined on 1st, 3rd, and 7th day. On the first day, ethanolic extract showed significant decrease in serum glucose level to 41.42% as compared to standard which was 44.50% at 5th h. Petroleum ether extract did not show significant decrease in serum glucose level. The ethanolic extract further reduced serum glucose level with respect to corresponding control, but the petroleum ether extract did not show any significant decrease in serum glucose level up to 7 days. The ethanolic extract reduced serum glucose level to 51.30% as compared to standard which was 63.11% on 7th day.

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