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R E S E A R C H A R T I C L E

Investigation for Antidiabetic Activity of Ethanolic Bark Extracts of *Viburnum Opulus*

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A B S T R A C T

In the presant work an attempt has been made to investigate the anti diabetic activity of the ethanolic extracts of the plant viburnum opulus, The results concluded that EEVO (500 mg/kg) have definite antidiabetic activity in Streptozocin induced Diabetic model and which is equipotent activity when compared with Atorvastatin treated groups. Further studies on this extract may lead to identify the possible mechanism of action and isolation of active principle from the same. *EEVO* have different medicinal properties and may able to treat diabetes & diabetics complications. Subjected to acute oral toxicity studies and found that the *EEVO* is safe to use up to the dose of 1000mg/kg. The *EEVO* was found to be in dose dependent way against alloxan induced diabetes in rats. The reduction of the elevated blood glucose levels in diabetic rats on treatment with the extract at two different concentrations confirmed that ethanolic extract of *EEV O*posses Antidiabetic activity & has shown significant effect when compared to Streptozocin administration.

Keywords: *viburnum opulus,* Antidiabetic activity

A R T I C L E I N F O

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CONTENTS

1. Introduction

Diabetes is a metabolic disorder which can be considered as a major cause of high economic loss which can in turn impede the development of nations. Moreover, uncontrolled diabetes leads to many chronic complications such as blindness, heart failure, and renal failure. In order to prevent this alarming health problem, the development of

research into new hypoglycaemic and potentially anti diabetic agents is of great interest. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of Indian traditional health care systems. In Indian systems of medicine most practitioners formulate and dispense their own recipes. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world. The current review focuses on herbal drug preparations and plants used in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses.

Advancement in science and technology have brought significant development in the field of toxicity testing. Improvement of the conventional methods through application of up-to-date techniques is the issue of the present day. In toxicity assessment of chemicals, there is no doubt that the best test species for humans are humans since accurate extrapolation of animal data directly to humans may not be guaranteed due to interspecies variation in anatomy, physiology and biochemistry. However, due to ethical reasons, such chemicals are to be tested using animal models before they are subjected to trials in humans. Viburnum opulus is a deciduous shrub growing to 4–5 m (13–16 ft) tall. The leaves are opposite, three-lobed, 5–10 cm (2–4 in) long and broad, with a rounded base and coarsely serrated margins; they are superficially similar to the leaves of some maples, most easily distinguished by their somewhat wrinkled surface with impressed leaf venation. The leaf buds are green, with valvate bud scales. The hermaphrodite flowers are white, produced in corymbs 4–11 cm (2–4 in) in diameter at the top of the stems; each corymb comprises a ring of outer sterile flowers 1.5–2 cm in diameter with conspicuous petals, surrounding a center of small (5 mm), fertile flowers; the flowers are produced in early summer, and pollinated by insects.

The fruit is a globose bright red drupe 7–10 mm diameter, containing a single seed. The seeds are dispersed by birds. The conventional acute toxicity test which involves the use of large numbers of animals is being replaced by alternative methods. The methods require that fewer numbers of animals or other models that do not require the use of animals (such as in-silico and in vitro approaches) are employed. In the screening of drugs, determination of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals) is usually aninitialstepinthe assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations (provides information on health hazards likely to arise from short-term exposure to drugs) and is one of the initial screening experiments performed with all compounds.

2. Materials and Methods

Materials used

Reduced nicotinamide adenine dinucleotide (NADH), Glutathione reduced are bought from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Hydrogen peroxide, Ethanol, 2,4-dinitro phenylhydrazine (DNPH), Dipotassium hydrogen phosphate, Potassium dihydrogen phosphate are bought from Merck, Mumbai, India.Azathioprine is bought from RPG Life sciences Pvt, Ltd, Hyd. Ascorbic acid is bought from Finar chemicals, Ahmedabad, India. Normal saline is bought from Claris life sciences. Ltd., Ahmedabad, India.

Methods

I. Collection and Authentification of Plant Material

The Aerial Parts of *viburnum opulus*were collected and authenticated

II. Extraction of Plant Material

The plant is grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Ethanol Extraction)[38]

In this work the cold extraction process was done with the help of ethanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of ethanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent

The filtrates (ethanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccator for 7 days.

III. Preliminary Phytochemical Screening

Preliminary phytochemical screening of the *viburnum opulus* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids as per the standard methods [40].

IV. Animals

Healthy Adult Male wistar rats of 8-10 weeks old with Average weight in the range of 150-180gms were selected. Animals are housed 4 per cage in temperature controlled ($27\ ^0C \pm 3\ ^0c$) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *ad libitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

V. Acute toxicity studies [41]

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organisation for Economic Co-operation and Development) guidelines no 423. Female Albino wistar rats (130-200 g) were taken for the study and dosed once with 2000 mg/kg of the extract. The treated animals were monitored for 14 days to observe general clinical signs and

symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, ¼ and 1/8 doses of 2000 mg/kg i.e. 500 mg/kg and 250 mg/kg were chosen for subsequent experimentation.

VI.Method of Induction

The systemic administration of the Streptozocin to rats results in elevation of diabeteswas induced in Wistar albino rats by single *i.v* injection of freshly prepared solution of Streptozocin (60 mg/kg) in physiological saline solution after overnight fasting for 18 h. rats will develop diabetes in 3 days due to the destruction of beta cells

VII. Experimental Study Design for Diabetic screening

Diabetic rats were divided in to five groups with each group four animals.

Group-I: Rats served as normal control group. given normal saline as treatmen.

Group-II: served as diabetic/disease control. given Streptozocin (60mg/kg.i.v.).

Group-III: Diabetic rats treated with *viburnum opulus*at a dose 250 mg/kg.

Group-IV: Diabetic rats treated with *viburnum opulus*at a dose of 500 mg/kg

Group V: Diabetic rats treated with Metformin (standard drug) at 450mg/kg[24].

The treatment was given for 14days and blood samples were collected at different intervals.

VIII. Collection of blood samples

Blood samples were collected from all the groups of animals at 0, 7,15th day intervals through puncture of retro orbital plexus and were centrifuged at 3000 revolutions per minute (rpm) for 15 minutes. Serum was separated and stored at -20^{0}c and then used for estimating blood glucose levels.

IX. Evalution Parameter

GLUCOSE[23] Method: GOD/POD method

Principle:

D-glucose + H_2O+ O_2 glucose oxidase (GOD) gluconic acid + H_2O_2 ⟶

H_2O_2 + 4-AAP +Phenol peroxidase (POD) Quinoneimine dye + H_2O ⟶

Procedure:

- Wavelength/filter : 505 nm (Hg 546 nm) / Green
- Temperature: 37^0 C / R.T.
- Light path : 1 cm
- Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T)

Addition Sequence	B (ml)	S (ml)	T (ml)
Glucose Reagent L1)	1.0	1.0	1.0
Distilled Water	0.01	--	--
Glucose Standard S)	--	0.01	--
Sample	--	--	0.01

Mix well and incubate at 37^{0}C for 10 min or at R.T. (25^{0}C) for 30 mins. Measure absorbances of the Standard (Abs.S) and Test Sample (Abs.T) compare these against the Blank within 60 mins.

X. Statistical Analysis

Results are expressed as Mean ± S.D .all the results were compared with control subject one-way analysis of variance (ANOVA), followed by the dunnet t-test using Graph Pad Prism Software 6 version. P Values < 0.05 were as considered statistically significant.

3. Results & Discussion

% Yield of ethanolic Extract from Aerial Parts of *EEVO* was found to be **12.27%**

Preliminary Phytochemical Screening

Table.no.1. Preliminary Phytochemical Screening

Phytochemical	Results
Steroid	Absent
Alkaloid	Present
Tannin	Slightly Present
Carbohydrate	Absent
Phenol	Absent
Flavonoid	Present
Saponin	Present

(+) Present. **(-)** Absent

Acute toxicity studies

As per (OECD) draft guidelines 423 adopted, male wistar rats were administered with *EEVO* and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Additional observations like behavioral changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern were also found to be normal. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma.

Glucose

Table 2: Effect of *EEVO (EEVO)* on serum glucose levels (mg/dl) in diabetic rats

Groups/Interval	0th Day	7th Day	15th Day
Normal	65.48±0.35	67.89±0.18	69.96±0.78
Diabetic control	185.65±0.64	188.64±0.65	189.67±0.95
EEVO (250mg/kg)	164.48±0.48	159.74±0.74	145.86±0.64
EEVO (500mg/kg)	142.65±0.46	138.65±0.71	135.75±0.48
Metformin (450mg/kg)	120.34±0.75	115.75±0.48	111.34±0.69

All the values of mean ± SD; n=3,

Discussion

The present study was aimed to evaluate the anti diabetic activity of *EEVO*. The activity was measured by estimating various biomarkers like blood glucose levels, in experimental rats.The *EEVO*has reported anti-microbial properties but the effect of the plant extract on antidiabetic, were not reported yet and so the plant was chosen for the study.Streptozocin forms an increased glucose levels that generates diabetes. Pretreatment with *EEVO*produced significant decrease in glucose levels indicating the protective effect of tissue. On Streptozocin treatment a dose dependent decrease in glucose levels were observed. Pretreatment with *EEVO*and metformin produced significant alteration in levels.

4. Conclusion

The results concluded that EEVO (500 mg/kg) have definite antidiabetic activity in Streptozocin induced diabetic model and which is equipotent activity when compared with metformin treated groups. Further studies on this extract may lead to identify the possible mechanism of action and isolation of active principle from the same. *EEVO* have different medicinal properties and May able to treat diabetes & diabetics complications. Subjected to acute oral toxicity studies and found that the *EEVO* is safe to use up to the dose of 1000mg/kg. The *EEVO* was found to be in dose dependent way against alloxan induced diabetes in rats. The reduction of the elevated blood glucose levels in diabetic rats on treatment with the extract at two different concentrations confirmed that ethanolic extract of *EEVO* posse's Antidiabetic activity & has shown significant effect when compared to Streptozocin administration.

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