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Research Article

Preliminary Phytochemical Analysis of Extract of *Spermadictyon suaveolens* and its Effect on Oral Glucose Tolerance in Streptozotocin Induced Diabetic Rats

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ABSTRACT

The preliminary phytochemical analysis of extract of *Spermadictyon suaveolens* was performed and its effect on oral glucose tolerance in streptozotocin induced diabetic rats studied. 1 week after the administration of streptozotocin, diabetic rats received herbal extract orally at dose 500 mg/kg body weight for 28 consecutive days. Oral glucose tolerance test was performed on overnight fasted rats on day 28. Blood samples were collected prior to glucose administration and at 30, 60, 90, 120 minutes after glucose loading and plasma glucose level was measured for all the samples. The preliminary phytochemical analysis of extract of roots of *Spermadictyon suaveolens* showed the presence of carbohydrates, flavonoids, glycosides, tannins and phenolic compounds. Extract of roots of *Spermadictyon suaveolens* improved oral glucose tolerance in streptozotocin induced diabetic rats.

Keywords: Streptozotocin, *Spermadictyon suaveolens*, OGTT, Diabetes.

INTRODUCTION

Increasing prevalence of obesity and physical inactivity, unhealthy lifestyles and many other factors are making diabetes - a global epidemic. It is reported that globally there were 382 million people with diabetes in the year 2013 and this figure is expected to hit 592 million by 2035[1,2]. Oral glucose tolerance test (OGTT) is useful in assessing the prevalence of diabetes and impaired glucose tolerance (IGT). In diabetes mellitus, carbohydrate metabolism is altered which can be studied by OGTT[3]. In response to oral glucose, the small intestine releases gastrointestinal factor which has influence on the metabolism of absorbed glucose through stimulation of insulin secretion and thus gut influences the metabolism of glucose in muscle and adipose tissue. Orally administered glucose acts as stimulus to insulin release and this stimulus plays significant role in the disposition of a glucose load and hence on glucose tolerance[4]. Therefore, oral glucose tolerance test is important in the study of anti-diabetic agent.

A number of plants are known to be used traditionally for the treatment of diabetes. The progress in the field of synthetic agents for the treatment of diabetes is significant but these treatments accompany the disadvantages like drug resistance, side effects and even toxicity[5]. Because of the natural origin and less side effects, herbal drugs are gaining popularity in the management of diabetes both in developing and developed countries[6].

The secondary metabolites such as alkaloids, flavonoids, tannins and other phenolic compounds are important bioactive components in plant having medicinal value[7]. Qualitative analysis of phytoconstituents therefore helps to understand the therapeutic effectiveness of the plant.

The plant *Spermadictyon suaveolens* Roxb. [Synonym: *Hamiltonia suaveolens* (Roxb.) Roxb.] belonging to family Rubiaceae is important for its antidiabetic activity[8]. In the present study, we performed the preliminary phytochemical analysis of extract of *Spermadictyon suaveolens* and its effect on oral glucose tolerance in streptozotocin induced diabetic rats studied.

MATERIALS AND METHOD

Plant material

The plant material was procured from Konkan region i.e. Dapoli, District: Ratnagiri, State: Maharashtra, India. The roots of *Spermadictyon suaveolens* were collected. The plant was authenticated at Blatter Herbarium, St. Xavier's College, Mumbai, India. The specimen matches with the Blatter Herbarium specimen number 11696 of H. Santapau.

Preparation of herbal extract

Roots of *Spermadictyon suaveolens* were washed and dried in air at room temperature. For extraction, roots were rubbed on stone with little distilled water to form a paste. Paste was dried in air at room temperature by spreading in petri plate in order to allow water to evaporate. The extract obtained was triturated using mortar and pestle. This powdered extract was suspended in distilled water using sodium carboxy methyl cellulose for oral administration. For qualitative analysis of phytoconstituents, extract in the

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powder form or other form used as per the requirement of individual test.

Chemicals

Standard reagents and chemicals of analytical grade were used. Metformin was obtained as a gift sample from Aarti Drugs Limited, Mumbai, India; Batch Number: MEF/15090169.

Animals

Male Sprague Dawley rats weighing 200-250 g were procured from Bharat Serum and Vaccines, Thane. Animals were maintained in the SVKM's Animal Facility, Vile Parle (W), Mumbai, under standard conditions (temperature 25^0 C ± 2^0 C, relative humidity 75% ± 5% and 12 hour light/dark cycle). Rats were provided with standard laboratory feed and water ad libitum. Rats were allowed to acclimatize for one week before the onset of the experiment[9]. Protocol for study was approved by Institutional Animal Ethics Committee, which follows guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). (Reference Number: CPCSEA/IAEC/BNCP/P-02)

Morphological study

Organoleptic study for plant roots was done. Various organoleptic characters like colour, odour, shape were assessed.

Qualitative analysis:

To identify the presence of phytoconstituents like carbohydrates, proteins, amino acids, fats and oils, steroid, glycoside, flavonoid, alkaloids, tannins and phenolic compounds the herbal extract was subjected to qualitative analysis.

Tests for Carbohydrates

Molisch's test (General test)

To 2-3 ml aqueous extract, few drops of alphanaphthol solution in alcohol were added and shaken. Concentrated sulphuric acid was added from sides of the test tube. Violet ring is formed at the junction of two liquids if carbohydrates are present.

Fehling's test

1 ml Fehling's A solution and 1 ml Fehling's B solution were mixed, boiled for 1 minute. Equal volume of test solution was added. Heated in boiling water bath for 5-10 minutes. Reducing sugars give first yellow, then brick red precipitate.

Benedict's test

Equal volume of Benedict's reagent and test solution were mixed in test tube and heated in boiling water bath for 5 minutes. Colour was observed. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

Tests for Proteins

Biuret test

To 3 ml test solution 4% sodium hydroxide and few drops of 1% copper sulphate solution were added. Protein gives violet or pink colour.

Millon's test

3 ml test solution and 5 ml Millon's reagent were mixed. White precipitate forms. After warming precipitate turns brick red or the precipitate dissolves giving red coloured solution if protein present.

Test for Amino Acids

Ninhydrin test (General test)

3 ml test solution and 3 drops of 5% Ninhydrin solution were heated in boiling water bath for 10 minutes. Amino acids give purple or bluish colour.

Tests for Fats and Oils

A thick section of drug was placed on glass slide. A drop of Sudan Red III reagent was added. After 2 minutes, washed with 50% alcohol. Mounted in glycerin and observed under microscope. Oil globules appear red if present.

Solubility test

Solubility was checked in benzene, chloroform as oils are soluble in benzene, chloroform.

Filter paper gets permanently stained with oils.

Tests for Steroid

Salkowski reaction

To 2 ml of extract, 2 ml chloroform and 2 ml concentrated sulphuric acid were added. Chloroform layer appears red and acid layer shows greenish yellow fluorescence if steroid present.

Liebermann – Burchard reaction

2 ml of extract was mixed with chloroform. 1-2 ml acetic anhydride and 2 drops concentrated sulphuric acid were added from the side of test tube. First red, then blue and finally green colour appears if steroid present.

Liebermann's reaction

3 ml extract and 3 ml acetic anhydride were mixed. Heated and cooled. Few drops of concentrated sulphuric acid added. Blue colour appears if steroid present.

Tests for Glycosides

General test

Test A

200 mg of drug extracted with 5 ml of dilute sulphuric acid by warming on a water bath. After filtering the acid extract was neutralized with 5% solution of sodium hydroxide. 0.1 ml of Fehling's solution A and B were added until it becomes alkaline (test with pH paper) and heated on a water bath for 2 minutes. The quantity of red precipitate formed was noted and compared with that of formed in test B.

Test B

200 mg of drug extracted with 5 ml of water instead of sulphuric acid. After boiling, equal amount of water was added as used for sodium hydroxide in the above test. 0.1 ml of Fehling's solution A and B were added until it becomes alkaline (test with pH paper) and heated on a water bath for 2 minutes.

The quantity of red precipitate formed was noted. The quantity of precipitate formed in Test B was compared with that of formed in Test A. If the precipitate in Test A is greater than in Test B then Glycoside may be present. Since Test B represents the amount of free reducing sugar already present in the crude drug, whereas Test A represents free reducing sugar plus those related on acid hydrolysis of any glycoside in the crude drug.

Tests for cardiac glycosides

Baljet's test

A thick section shows yellow to orange colour with sodium picrate.

Keller-Killiani test: To 2 ml extract, glacial acetic acid, one drop 5% ferric chloride and concentrated sulphuric acid were added. Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish green.

Tests for anthraquinone glycosides

Borntrager's test for anthraquinone glycosides

To 3 ml of extract, dilute sulphuric acid was added, boiled and filtered. To cold filtrate, equal volume chloroform was added and shaken. Organic solvent was separated. Ammonia was added. Ammonical layer turns pink or red if test is positive.

Modified Borntrager's test for C-glycosides

To 5 ml extract, 5 ml 5% ferric chloride and 5 ml dilute hydrochloric acid were added. Heated for 5 minutes on boiling water bath. Cooled, benzene was added and shaken. Separated the organic layer and equal volume dilute ammonia was added. For positive test, ammonical layer shows pinkish red colour.

Test for saponin glycosides

Foam test: Drug extract was vigorously shaken. Persistent foam is observed.

Tests for cyanogenetic glycoside

Grignard reaction or sodium picrate test

A filter paper strip was soaked first in 10% picric acid and then in 10% sodium carbonate and dried. In a conical flask, moistened powdered drug was placed. It was corked and the above filter paper strip was placed in the slit in cork. The filter paper turns brick red or maroon.

To dry drug powder 3% aqueous mercurous nitrate solution was added. Metallic mercury forms.

Tests for Flavonoids

Shinoda test

To dry powder, 5 ml 95% alcohol, few drops concentrated hydrochloric acid and 0.5 gram magnesium turnings were added. Orange, pink, red to purple colour appears.

(Note: Add t-butyl alcohol before adding the acid to avoid accidents from a violent reaction and to dissolve into the upper phase. By using zinc instead of magnesium, only flavanols give a deep red to magenta colour while flavanones and flavonols give weak pink to magnetic colours or no colour.)

Sulphuric acid test

On addition of sulphuric acid flavones and flavonols dissolve into it and give a deep yellow solution. Chalcones and aurones give red or red-bluish solutions. Flavanes give orange to red colours.

To small quantity of residue, lead acetate solution was added. Yellow coloured precipitate is formed.

Addition of increasing amount of sodium hydroxide to residue shows colouration, which decolourises after addition of acid.

Tests for Alkaloids

The extract was evaporated. To residue, dilute hydrochloric acid was added, shaken and filtered. With filtrate, following tests were performed.

Dragendorff's test

To 2-3 ml filtrate, few drops of Dragendorff's reagent were added. Orange brown precipitate is formed if alkaloids are present.

Mayer's test

To 2-3 ml filtrate, few drops of Mayer's reagent were added. Precipitate is formed if alkaloids are present.

Hager's test

2-3 ml filtrate with Hager's reagent gives yellow precipitate if alkaloids are present.

Wagner's test

2-3 ml filtrate with few drops Wagner's reagent gives reddish brown precipitate if alkaloids are present.

Tests for Tannins and Phenolic Compounds

2-3 ml of extract with few drops of 5% ferric chloride solution gives deep blue-black colour.

2-3 ml of extract with few drops of lead acetate solution gives white precipitate.

2-3 ml of extract with few drops of gelatin solution gives white precipitate.

Extract shows decolouration of bromine water.

2-3 ml of extract with few drops of acetic acid solution shows red colour.

2-3 ml of extract with few drops of potassium dichromate shows red precipitate.

2-3 ml of extract with few drops of potassium permanganate solution shows decolouration.[10]

Induction of diabetes in experimental animals

For induction of diabetes mellitus, a single intraperitoneal injection of STZ (Streptozotocin) in 0.1 M sodium citrate buffer (pH 4.5) was given to overnight fasted rats at dose 55 mg/kg body weight. Animals were provided with 10% glucose solution after injection of streptozotocin to decrease mortality in animals. 3 days later, plasma glucose levels were checked. For the study, animals with plasma glucose levels more than 200 mg/dL were selected.

Experimental design

Rats were kept for 1 week after injection of streptozotocin to stabilise the diabetic conditions and permanent hyperglycaemia before starting treatment. In the experiment, Sprague Dawley rats were randomly divided in 4 groups with 6 animals in each group (n=6). Rats in normal control and diabetic control groups received sodium carboxy methyl cellulose in distilled water. One group of diabetic rats treated with extract of roots of *Spermadictyon suaveolens* at dose 500 mg/kg body weight and one group of diabetic rats treated with metformin at dose 100 mg/kg body weight. Each rat of each group received vehicle or respective treatment for 28 consecutive days as a single daily dose orally. OGTT (Oral Glucose Tolerance Test) was performed on overnight fasted rats. On day 28, rats in all groups were given glucose (2 g/kg body weight; orally). Blood samples were collected prior to glucose administration and at 30, 60, 90, 120 minutes after glucose loading and plasma glucose level was measured for all the samples[11,12]. Estimation of glucose was done using commercially available diagnostic kit in ErbaChem 7 biochemistry analyser.

Statistical analysis

Data are expressed as mean ± SEM. OGTT data were analysed using two way ANOVA (Analysis of Variance) test followed by Bonferroni test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Table 1: Effect of treatment with extract of roots of *Spermadictyon suaveolens* on plasma glucose in OGTT in STZ-induced diabetic rats (Plasma glucose level is expressed as mg/dL).

Group	0 Minute	30 Minute	60 Minute	90 Minute	120 Minute
Normal control	110.22 ± 2.74	139.83 ± 2.63	151.16 ± 3.22	131.35 ± 2.98	122.5 ± 3.97
Diabetic control	373 ± 10.9	455.02 ± 5.25	480 ± 4.16	547 ± 4.33	475 ± 4.16 a
Extract (500 mg/kg body weight)	160.03 ± 7	230.08 ± 4.62	320 ± 3.65	287.06 ± 3.38	190.03 ± 4.11 b
Metformin (100 mg/kg body weight)	165.03 ± 5.37	347.01 ± 5.66	340 ± 4.88	320 ± 5	300 ± 4 b

The values are expressed as Mean ± SEM at n=6; a: $p < 0.001$ compared to normal control; b: $p < 0.001$ compared to diabetic control

Morphological study revealed that the root of *Spermadictyon suaveolens* is buff white in colour with faint and characteristic odour. The shape of root is cylindrical. The preliminary phytochemical analysis of extract of roots of *Spermadictyon suaveolens* showed the presence of carbohydrates, reducing sugars and starch. Cardiac glycosides, saponin glycosides, cyanogenetic glycoside were present. Flavonoids, tannins and phenolic compounds were present.

Table 1 shows plasma glucose levels at particular intervals during OGTT. In non-diabetic group little increase in plasma glucose level was seen on administration of glucose and the level came to normal at the end of 120 minutes. In diabetic control, significant increase in plasma glucose level was seen upon administration of glucose which did not decrease significantly at the end of 2 hours. At the end of 2 hours, plasma glucose level was significantly low in the treatment groups as compared to diabetic control group.

Alteration in the carbohydrate metabolism during post glucose administration can be identified by glucose tolerance test[12]. Treatment with extract of roots of *Spermadictyon suaveolens* lowered the plasma glucose level in the oral glucose tolerance test. This suggests that rats receiving above treatment have better glucose utilization capacity. The glucose lowering activity of extract of roots of *Spermadictyon suaveolens* could be due to presence of flavonoids. Flavonoids act as antioxidant and contribute to anti-diabetic property.

CONCLUSION

Extract of roots of *Spermadictyon suaveolens* improves oral glucose tolerance in streptozotocin induced diabetic rats**.** As the phytochemical analysis revealed the presence of constituents which contribute to antidiabetic activity, the extract of roots *of Spermadictyon suaveolens* is important for its antidiabetic activity and will be useful in the treatment of impaired oral glucose tolerance.

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CONFLICT OF INTEREST NOTIFICATION

The authors declare that there are no conflicts of interest.

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