EVALUATION OF ANTIDIABETIC ACTION OF ARAUCARIA COLUMNARIS LEAF EXTRACTS

Devendra Kumar Rawat[1], Arun Kumar Sharma[2], Vishal Shrivastava[3], Kehar Singh Dhakad[4]

[1]Student, Department of Pharmacology, School of Pharmacy, LNCT University, Bhopal, MP, India
[2]Student, Department of Pharmacology, School of Pharmacy, LNCT University, Bhopal, MP, India
[3]Assistant Professor, Department of Pharmacology, School of Pharmacy, LNCT University, Bhopal, MP, India
[4]Assistant Professor, Department of Pharmacology, School of Pharmacy, LNCT University, Bhopal, MP, India

ABSTRACT

The leaf bearing branchlets are narrow at the petiole, broaden towards the middle and narrow down towards the apex. The ability of different solvents to extract phytochemicals from leaves followed the order: methanol>water>ethyl acetate>benzene. The preliminary qualitative phytochemical analysis suggests the presence of saponin glycosides, cardiac glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaves. The total phenolic content of benzene, ethyl acetate, methanolic and aqueous extract of Araucaria columnaris were 5.18±0.91, 8.97±0.17, 63.22±0.48 and 38.24±0.63 GAE mg/g, respectively. All the extracts were subjected to in vitro determination of antioxidant potential using DPPH scavenging method. The IC50 value of the DPPH scavenging potential for benzene and ethyl acetate was found to be more than 250µg/mL whereas for methanol and aqueous extracts was found to be 136.6 and 200.2 µg/mL respectively. Alloxan induced diabetes model was used for determining the antidiabetic potential of the methanolic and aqueous extracts. The aqueous extract was able to lower the blood glucose more in comparison to the methanolic extract comparable to the standard drug glibenclamide.

Keywords: - *Araucaria Columnaris; Alloxan induced diabetic model; Antidiabetic; Wistar Rat.*

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia that may result from defects in insulin secretion or action or both. Diabetes occurs when the β cells of the islets of Langerhans in the pancreas are unable to produce enough insulin or when the body is not able to utilize the insulin that has been produced. The deficient insulin action leads to abnormalities mainly in carbohydrate and fat metabolism. The deficiency in carbohydrate metabolism leads to accumulation of sugar in blood (hyperglycemia). As energy production decreases in the body, lipolysis increases and liponeogenesis is decreased. This leads to the loss of adipose tissues in the body and eventually causes weight loss [1]. A number of medicinal and aromatic plants have been evaluated for their antidiabetic potential. The source of using plants in the treatment of diabetes has been documented in the Ayurveda since ancient times [2]. Araucaria is a genus of coniferous trees in the Araucariaceae family [3]. The plants of Araucaria species have been known to possess emollient, antiseptic, antiemetic, wound healing actions. They have been used in toothache, amenorrhea, respiratory infections and rheumatism. Some phytochemical isolated from various plants of the genus include flavonoids ginkgetin, Bilobetin, II-7-O-methyl-robustaflavone, cupressuflavone, Cabreuvin, Irisolidone, Lignans, Abietanes and terpenes like geraniolene, limonene and Labdane [4-5].

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant Material

The leaves of A. columnaris were collected from the local places of Bhopal, Madhya Pradesh. The authenticated plant leaves were washed with distilled water, dried under shade and powdered using a blender at low speed. The powdered leaves were stored in air tight container until taken for use [6].

2.2. Extraction of leaves

The powdered leaves were used for the extraction process. 500 g of powder was evenly packed in the extractor of the Soxhlet apparatus and extracted successively with various solvents of increasing polarity including benzene, ethyl acetate, and methanol by hot continuous extraction process for about 27 h. The aqueous extraction was carried out by cold maceration process after completion of the solvent extraction process. The extracts were filtered while hot through Whatman filter paper to remove any impurity. The extracts were concentrated by distillation to reduce the volume to 1/10. The concentrated extracts were transferred to 100 ml beaker and the remaining solvents were evaporated on water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture [7].

2.3. Preliminary phytochemical screening [8]

All the extracts were evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in them.

2.3.1. Test for Alkaloids

2.3.1.1. Mayer's Test: To a few ml of plant sample extract, two drops of Mayer's reagent was added along the sides of test tube.

2.3.1.2. Wagner's test: A few drops of Wagner's reagent were added to few ml of plant extract along the sides of test tube.

2.3.1.3. Hager's test: A few drops of Hager's reagent were added to few ml of plant extract along the sides of test tube.

2.3.1.4. Dragendroff's Test: A few drops of Dragendroff's reagent were added to 1 ml of each extract.

2.3.2. Test for Glycosides

2.3.2.1. Saponin glycosides

2.3.2.1.1. Froth test: 1 ml solution of the extract in water was placed in a test tube and shaken vigorously.

2.3.2.2. Cardiac glycosides

2.3.2.2.1. Kedde's test: The extract was extracted with chloroform and evaporated to dryness. One drop of 90% alcohol and 2 drops of 2% 3, 5-dinitro benzoic acid (3, 5-dinitro benzene carboxylic acid Kedde's reagent) in 90% alcohol are added to the above residue. The solution is made alkaline with 20% sodium hydroxide solution.

2.3.3. Test for Tannins and phenolic compounds

2.3.3.1. Gelatin test: To the extract was added 1% gelatin solution containing 10% sodium chloride.

2.3.3.2. Ferric chloride test: To the extract was added a freshly prepared solution of ferric chloride.

2.3.3.3. Vanillin hydrochloride test: Test solution of the extract was treated with few drops of vanillin hydrochloride reagent.

2.3.3.4. Alkaline reagent test: Test solution of the extract was treated with sodium hydroxide solution.

2.3.4. Test for Flavonoids

2.3.4.1. Shinoda test: To the test solution of the extract, few fragments of magnesium ribbon were added and conc. hydrochloric acid was mixed drop wise to it.

2.3.4.2. Zinc hydrochloride reduction test: To the test solution a mixture of zinc dust and conc. hydrochloric acid was added.

2.3.5. Test for Proteins and amino acids

2.3.5.1. Millons test: Test solution of the extract was allowed to react with 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid).

2.3.5.2. Ninhydrin test: The solution of extract was boiled with 0.2% solution of ninhydrin.

2.3.6. Test for Sterols and triterpenoids

2.3.6.1. Libermann Burchard test: Extract was treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added from the sides of the test tube.

2.3.6.2. Salkowski test: The extract was dissolved in chloroform and a few drops of conc. sulphuric acid were added to it. The mixture was shaken well and allowed to stand for some time.

3. TOTAL PHENOLIC CONTENT [9]

The extraction of phenolic compounds was based on a modified method by Hsu et al. Briefly 5 g dried powder of leaves was mixed with 80 mL of methanol and kept overnight. The suspension was filtered through a qualitative cellulose filter paper and the filtrate was diluted to 100 mL with methanol. The solution was stored at 4°C in amber bottles and served as the stock solution (50 mg/mL) for subsequent analyses. For total phenolic content determination, 200 μL of sample was mixed with 1.4 mL purified water and 100 μL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 μL of 20% Na_2CO_3 aqueous solution was added and the mixture allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. The control solution contained 200 μL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

4. DETERMINATION OF ANTIDIABETIC ACTIVITY [10]

The in vivo evaluation of antidiabetic activity of the extracts was carried out in Wistar rats weighing between 150–180 g by alloxan induced diabetes model. The animals were housed in clean polypropylene cages with 12 h light and dark cycle at a temperature of 27-29°C and a relative humidity of 62 to 65%. The animals had access to food and water ad libitum. The animals were acclimatized to laboratory condition for one week before starting the experiment. The animals were fasted for at least 12 h before onset of each activity. Animals were fasted 18 h prior to the administration of freshly prepared alloxan (150 mg/kg i.p.) dissolved in 0.3% CMC solution. After 48 h, the animals were divided in various groups of six rats each (n = 6).
Group I (Normal): treated with 0.3% CMC solution (0.5 ml/100 g), orally single dose up to seven days.

Group II (Diabetic control): treated with alloxan (150 mg/kg, i.p) + 0.3% CMC solution (0.5 ml/100 g), single dose up to seven days.

Group III (Standard): treated with alloxan (150 mg/kg, i.p) + glibenclamide (10 mg/kg p.o.), single dose up to seven days.

Group IV: treated with alloxan (150 mg/kg, i.p) + methanolic extract (100 mg/kg), single dose up to seven days.

Group V: treated with alloxan (150 mg/kg, i.p) + aqueous extract (100 mg/kg), single dose up to seven days.

Blood samples were collected from the tail vein on 0 h, 1 h, 2 h, 4 h, 5 h and 7th day for the measurement of blood glucose level. The blood glucose level was determined by digital glucometer (Gluco check).

5. Statistical Analysis

The results of pharmacological studies were expressed as mean ± S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one-way ANOVA (analysis of variance) followed by Dunnett's multiple comparison Test. The result was considered statistically significant when P- value less than 0.001 ($P<0.001$) vs control.

6. RESULTS AND DISCUSSIONS

The present work focused on preparing successive solvent extracts of Araucaria columnaris and evaluating its antidiabetic action in alloxan induced diabetic rats. The results obtained from the investigation are presented:

6.1. Extraction Yields: The extraction yield of the leaf using different solvents i.e. methanol>water>ethyl acetate>benzene is 38.6>17.6>11.8>4.1, respectively.
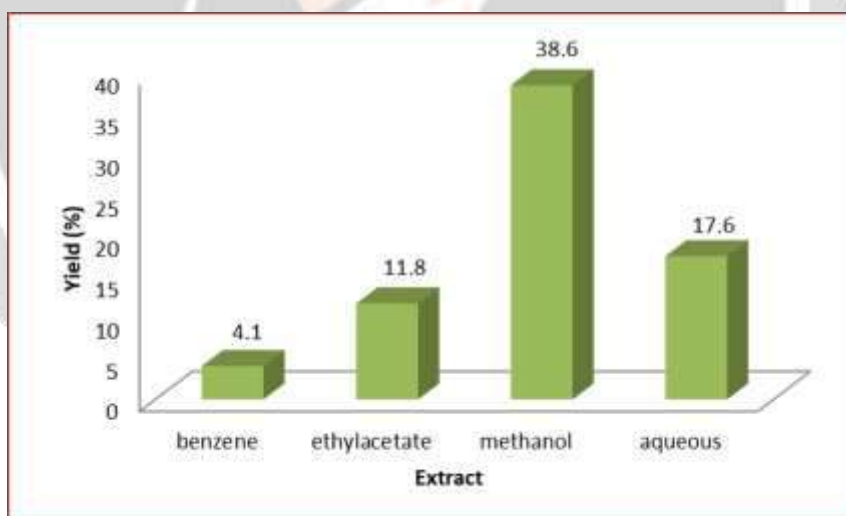

Chart 1.   **Extraction yields of different solvents**

6.2. Evaluation of Antidiabetic Activity

Alloxan induced diabetes is a widely accepted model for the evaluation of antidiabetic potential of drugs on rats and mice. Alloxan is a β-cytotoxin and causes an enormous destruction of β-cells of the islets of Langerhans, resulting in reduced synthesis and release of insulin. The function of the insulin system is suppressed, leading to hyperglycemia and eventually to death. The intraperitoneal injection of alloxan in Wistar rats produces hyperglycemia, impaired glucose tolerance and insulin resistance.

The results show that the glucose levels increase in alloxan treated rats as compared to the control while the extracts were able to reduce the blood glucose levels over time. The results reveal that the level of blood glucose in the control animal reduced by a small amount on the 7th day of testing whereas continual treatment with alloxan increased the levels of glucose tremendously. All the treated animals exhibited lowering of blood glucose with glibenclamide showing the maximum reduction. The aqueous extract was able to lower the blood glucose more in comparison to the methanolic extract.
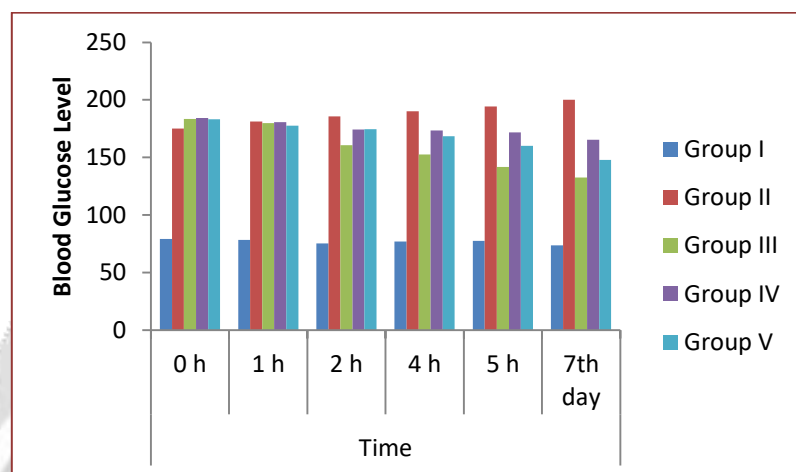
Chart 2.   **Comparative representation of the antidiabetic action**

7. CONCLUSION

The objective of the present study was to assess the antidiabetic potential of different leaf extract of Araucaria columnaris using the animal model. The results obtained led to the conclusion that Araucaria columnaris leaves are a rich source of potential antioxidants. The high phenolic content in the leaf extracts was vital for the antidiabetic activity exhibited by the plant. Further investigation of the mechanism by which the antidiabetic action occurs would be carried out using in vitro enzymatic models.

8. ACKNOWLEDGEMENT

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