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EXTRACTION, PHTOCHEMICAL AND ANTIDIABETIC EFFECT OF ROOTS EXTRACT OF *PLUMBAGO INDICA*

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Abstract
Plumbago indica (Plumbaginaceae) named "agnichita" belonging to the family Plumbaginaceae. The family is sometimes referred to as the leadwort family or the plumbago family. Most species in this family are perennial herbaceous plants, but a few grow as shrubs. In present investigation aim to evaluate the antidiabetic effect of roots extract of Plumbago indica. In our study, there was decrease in blood glucose level was found in all treatment groups during study. Progressive decrease in blood glucose level was found in all treatment groups during study. At the end of experiment Glibenclamide 500 mcg/kgp.o., Plumbago indica 200 and 300 mg/kg/p.o. (110.00 ± 6.50; 116.00 ± 6.00 and 118.00 ± 5.50) treated group blood glucose level was decrease significantly ($p<0.05$) at 21st days, respectively. Impaired carbohydrate metabolism and developing insulin resistance is the main metabolic disorder in non- insulin dependent diabetes mellitus leading to hyperglycemia. Altered digestion and absorption of dietary carbohydrate, depletion of glycogen storage, increased gluconeogenesis, β-cell dysfunction, insulin resistance of peripheral tissue and defect in insulin signaling pathways are important causes of hyperglycemia.

Key words: *Plumbago indica, Extraction, Phytochemical, Antidiabetic activity,*

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INTRODUCTION:

Diabetes mellitus is a set of disorders that cause hyperglycemia and glucose intolerance as a result of a lack of insulin, defective insulin function, or both [1]. Failures in the regulatory systems for the storage and mobilisation of metabolic fuels, such as carbohydrate, lipid, and protein catabolism and anabolism, are caused by incorrect insulin synthesis, insulin action, or both, resulting in such challenges [2,3].

Hyperglycemia is intricately related to physiological and behavioural responses. When the brain senses hyperglycemia, nerve signals are sent to the pancreas and other organs to lessen the impact. In type 2 diabetes these mechanisms break down, with the consequence that the two main pathological defects in type 2 diabetes are impaired insulin secretion through a dysfunction of the pancreatic β-cell, and impaired insulin action through insulin resistance. When insulin resistance is common, the majority of -cells alter, increasing insulin supply while compensating for the excessive and abnormal demand. Although plasma insulin concentrations are normally enhanced in absolute terms (both fasting and meal stimulated), they are insufficient to sustain normal glucose homeostasis "relative" to the degree of insulin resistance. Given the close link between insulin secretion and hormone action sensitivity in the complex management of glucose homeostasis, it's nearly difficult to disentangle the contributions of each to the etiopathogenesis of DM2 [4].
Insulin resistance and hyperinsulinemia eventually lead to impaired glucose tolerance. Except for maturity onset diabetes of the young (MODY), the mode of inheritance for type 2 diabetes mellitus is unclear. MODY, inherited as an autosomal dominant trait, may result from mutations in glucokinase gene on chromosome 7p. MODY is defined as hyperglycemia diagnosed before the age of twenty-five years and treatable for over five years without insulin in cases where islet cell antibodies (ICA) are negative [5].

In the last several decades, eco-friendly, bio-friendly, cost-effective, and usually safe plant-based pharmaceuticals have moved from the fringes to the mainstream, thanks to increased research in the area of traditional medicine. Medicinal plants have long been a significant resource for researchers looking for new ways to cure human illnesses. Several plants have traditionally been used for diabetic therapy. Numerous studies have also found that many plants have anti-diabetic effects.

There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycaemic agents. There are numerous traditional medicinal plants reported to have hypoglycemic properties such as *Allium sativum, Azadirachta indica*, *Vinca rosea* (Nayantara), *Momordica charantia* (Bitter ground), *Ocimum santum*. Many of these are less effective in lowering glucose levels in severe diabetes.

Plumbago indica (Plumbaginaceae) named "agnichita" belonging to the family Plumbaginaceae. The family is sometimes referred to as the leadwort family or the plumbago family. Most species in this family are perennial herbaceous plants, but a few grow as shrubs. In present investigation aim to evaluate the antidiabetic effect of roots extract of *Plumbago indica.*

MATERIAL AND METHODS:

Collection of plant material

Roots of *Plumbago indica* were collected from local area of Bhopal (M.P.) in the month of February, 2022.

Extraction of plant material

40.5 gram of dried powdered of roots of *Plumbago indica* has been extracted with aqueous using maceration for 48 hrs, filtered and dried using vaccum evaporator at 40^0C [6,7]

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening

Phytochemical examinations were carried out for all the extract as per the standard methods [8].

1. **Detection of alkaloids:** Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins and amino acids

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

6.6 Quantitative study of bioactive compound

6.6.1 Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method [9]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Estimation of Total protein content

1 ml of each BSA (Bovine serum albumin) working standard 50-250 µg/ml or test in test tubes. The test tube with 1 ml distilled water was serving as blank. Added 4.5 ml of reagent I and incubated for 10 minutes. After incubation added 0.5 ml of reagent II and incubated for 30 minutes. Measure the absorbance at 660 nm and plot the standard graph [10].

***In-vitro* antioxidant activity using nitric oxide method**

Nitric oxide was produced from sodium nitroprusside and the Griess reagent was measured. Sodium nitroprusside spontaneously produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitric ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen resulting in decreased nitric oxide manufacturing [10]. Sodium nitroprusside (10 mmol / L) was mixed with various extract concentrations in phosphate buffer saline (PBS) and incubated at 25°C for 150 min. Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) was added to the specimens. The chromophore absorbance created during the diazotization of sulphanilamide nitrite and subsequent coupling with napthylethyleneediamine was read at 546 nm and referred to the absorption of conventional ascorbic

acid solutions treated in the same manner with Griess reagent as a positive control. The inhibition proportion was evaluated using the following formula:

$$\text{Radical scavenging activity (\%)} = (A_{control} - A_{test})/A_{control} \times 100$$

Where Acontrol is the absorption (without extract) of the control and where Atest is the absorption in the presence of the extract / standard.

***In vitro* antimicrobial activity of roots of *Plumbago indica* extract**

Well diffusion method

The well diffusion method was used to determine the antibacterial activity of the extract prepared from the roots of *Plumbago indica* using standard procedure [11]. There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

***In vivo* anti-diabetic studies**

Animal grouping for anti-diabetic studies

Rats will be divided into different groups, each group consisting of six animals. After overnight fasting (deprived of food for 16 hours had been allowed free access to water) diabetes was induced in group II-VI by intraperitoneal injection of STZ dissolved in 0.1M sodium citrate buffer at pH 4.5, at a dose of 55mg/kg body weight. The control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug- induced hypoglycemia1. Diabetes status was confirmed by estimating blood glucose levels after 72 hours of STZ injection. Animals showing fasting blood glucose levels above 250 mg/dl were selected for study.

Table 1: Animal grouping for anti-diabetic studies on hydroacoholic extract of *Plumbago indica*

Group	Treatment	No. of Animals (n)
Group –I	Normal control received normal saline	6
Group -II	Diabetic control received normal saline	6
Group –III	Diabetic rats received Glibenclamide orally at dose of 500 mcg/kg b.wt for 14 days	6
Group –IV	Diabetic rats received Hydroacoholic extract of *Plumbago indica* (200 mg/kg/day p.o.)	6
Group -V	Diabetic rats received Hydroacoholic extract *Plumbago indica* (300 mg/kg/day p.o.)	6
Total No. of animal used for the study		30

RESULTS AND DISCUSSION:

This antihyperglycemic effect of hydroacoholic extract of *Plumbago indica* on the STZ-induced diabetic rats suggests that its main mechanism may not be due to stimulating insulin release from pancreatic cells, but may exert a direct action by promoting glucose utilization by peripheral tissues.

Intraperitoneal administration of STZ effectively induced diabetes mellitus in normal rats as reflected by blood glucose level and body weight loss compared with normal rats. The results demonstrate that *Plumbago indica* have antidiabetic activity as evaluated reduced the blood glucose level in STZ - induced diabetic rats. In our study, there was decrease in blood glucose level was found in all treatment groups during study. Progressive decrease in blood glucose level was found in all treatment groups during study. At the end of experiment Glibenclamide 500 mcg/kgp.o., *Plumbago indica* 200 and 300 mg/kg/p.o. (110.00 ± 6.50; 116.00 ± 6.00 and 118.00 ± 5.50) treated group blood glucose level was decrease significantly ($p<0.05$) at 21st days, respectively. Impaired carbohydrate metabolism and developing insulin resistance is the main metabolic disorder in non- insulin dependent diabetes mellitus leading to hyperglycemia. Altered digestion and absorption of dietary carbohydrate, depletion of glycogen storage, increased gluconeogenesis, β-cell dysfunction, insulin resistance of peripheral tissue and defect in insulin signaling pathways are important causes of hyperglycemia.

However, changes in initial and final body weight of normal control and experimental groups are shown in Table. Marked body weight loss was observed in diabetic rats.

The data obtained from this study showed that the treatment of *Plumbago indica* protects the diabetic rats from massive body weight loss, when given orally. *Plumbago indica* treated rats showed a

recovery in final body weight which was close to that of normal control rats. Moreover, body weights of animals in all groups were performed at the initial and end of the study. Body weight of animals was significantly ($p< 0.05$) maintained in all treatment groups (Glibenclamide 500 mcg/kg p.o., *Plumbago indica* 200 and 300 mg/kg/p.o., 174.40±8.26; 183.00±5.50 and 180.00 ± 9.70) during study as compared to control group (204.18±6.83).

Table 2: Phytochemical screening of *Plumbago indica* extract

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Dragendroff's test Wagner's test Mayer's test Hager's test	 -ve -ve -ve -ve
2.	**Glycosides** Legal's test	 +ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve -ve
4.	**Phenolics** $Fecl_3$	 -ve
5.	**Amino acids** Ninhydrin test	 -ve
6.	**Carbohydrates** Fehling's test	 -ve
7.	**Tannins** Gelatin test	 -ve
8.	**Proteins** Xanthoproteic test	 +ve
9.	**Saponins** Foam test	 +ve
10.	**Diterpenes** Copper acetate test:	 -ve

Table 3: Total bioactive constituents in *Plumbago indica*

S. No.	Extract	Total Flavonoid content (mg/100mg)	Total protein content (mg/100mg)
1.	Hydroalcoholic extract	0.741	0.365

Table 4: % Inhibition of ascorbic acid and extract of *Plumbago indica* using NO method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	20	43.63	14.51
2	40	67.24	31.76
3	60	77.42	38.28
4	80	80.56	42.63
5	100	84.57	52.47
IC 50		16.55	92.41

Table 5: Antimicrobial activity of *standard drug* against selected microbes

S. No.	Name of drug	Microbes	Zone of inhibition		
			10 µg/ml	20 µg/ml	30 µg/ml
1	Ofloxacin	*Streptococcus mutans*	12±0.15	15±0.13	17±0.19
2	Fluconazole	*Candida albicans*	26±0.47	30±0.74	32±0.5

Table 6: Antimicrobial activity of hydroalcoholic extract of *Plumbago indica* against selected microbes

S. No.	Name of microbes	Zone of inhibition		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Streptococcus mutans*	8±0.47	12±1.24	16±2.35
2.	*Candida albicans*	6±0	8±0.5	10±0.94

***(n=3, mean± SD)**

Table 7: Mortality, Neurological Behavior and signs of toxicity of Rats after acute oral administration of Hydroacoholic extract of *Plumbago indica*

Groups	Dose	Mortality	Neurological Behavior	Sign of toxicity
Normal control group	Normal saline	Nil	Normal	No sign of Toxicity
Plumbago indica Treated group	1000 mg/kg b.wt	Nil	Normal	No sign of Toxicity
Plumbago indica Treated group	1500 mg/kg b.wt	Nil	Normal	No sign of Toxicity
Plumbago indica Treated group	2000 mg/kg b.wt	Nil	Normal	No sign of Toxicity
Plumbago indica Treated group	2500 mg/kg b.wt	Nil	Normal	No sign of Toxicity
Plumbago indica Treated group	3000 mg/kg b.wt	Nil	Normal	No sign of Toxicity

Table 8: Mean body weight change

Group	Drug	Dose	Body weight (g)	
			Onset of study	End of study
I	Normal Control	Normal saline	184.15±6.83	204.18±6.83
II	Diabetic Control	Normal saline	194.20± 10.00	174.00±10.00
III	Glibenclamide	500 mcg/kg p.o.	204.22± 8.26	174.40±8.26
IV	Extract of *Plumbago indica* (EPI)	200 mg/kg p.o.	214.10± 5.50	183.00±5.50
V	Extract of *Plumbago indica* (EPI)	300 mg/kg p.o.	211.10± 5.00	180.00 ± 9.70

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. .control group respectively (One-way ANOVA followed by Dennett's test).

Table 9: Antidiabetic activity of hydroacoholic extract of *Plumbago indica* on blood glucose level in STZ-induced diabetic rats

Groups	Treatment	Dose	Blood glucose (mg/dl)		
			Days		
			0	8	21
I	Normal Control	Normal saline	78.00 ± 4.00	82.00 ± 4.00	91.00 ± 4.00
II	Diabetic Control	Normal saline	296.00 ± 7.00	375.00 ± 7.00	396.00± 7.00
III	Glibenclamide (STD)	500 mcg/kg p.o.	245.00± 6.50	135.00 ± 6.50	110.00 ± 6.50
IV	Extract of *Plumbago indica* (EPI)	200 mg/kg p.o.	260.00± 6.00	152.10 ± 6.00	116.00 ± 6.00
V	Extract of *Plumbago indica* (EPI)	300 mg/kg p.o.	265.00 ± 5.50	155.00 ± 5.50	118.00 ± 5.50

Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at $p<0.05$ vs.negative control group respectively (One-way ANOVA followed by Dunnett's test).

CONCLUSION:

The data obtained from this study showed that the treatment of *Plumbago indica* protects the diabetic rats from massive body weight loss, when given orally. *Plumbago indica* treated rats showed a recovery in final body weight which was close to that of normal control rats. Moreover, body weights of animals in all groups were performed at the initial and end of the study. Body weight of animals was significantly ($p< 0.05$) maintained in all treatment groups (Glibenclamide 500 mcg/kg p.o., *Plumbago indica* 200 and 300 mg/kg/p.o., 174.40±8.26; 183.00±5.50 and 180.00 ± 9.70) during study as compared to control group (204.18±6.83).

Dehydration and loss of body weight have been associated with diabetes mellitus. In diabetic rats, increased food consumption and decreased body weight were observed. This indicates the polyphagic condition and loss of weight due to excessive breakdown of tissue proteins. The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins.

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