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## Phytochemical analysis and Antidiabetic activity of *Withania somnifera* and *Cnidium monnieri* using Animal Models

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### ABSTRACT

Type 2 diabetic is a chronic, non-contagious disease with serious complications. The progression of excess weight leads to the increasing incidence among populations of diabetes mellitus type 2. In form 2 diabetes mellitus instances the median input of obesity is 90%. Moreover, around 174 million individuals worldwide are subject to glucose intolerance, the most prevalent being obesity and metabolic syndrome. There are many therapeutic plants in the medical field and out of these *Withania somnifera* and *Cnidium monnieri* are effective against many disorders such as allergy reactions, dermatophytes, many types of bacterial and fungal infections, osteoporotic, weakness, coldness, and skin-related disorders. This study aimed to check out the hypoglycemic, obesity prevention, cytotoxic & hepatoprotective activities of these two plants. The animals were divided into 7 groups, the first group was a normal controlled group and second group was intoxicated. The third group was treated with standard drugs Glucophage and Mevastatin. Rats of fourth, fifth, sixth, and seventh group was treated with extracts of *Withania somnifera* and *Cnidium monnieri* in high and low concentrations. As expecting these both gave positive results showing the significant decrease in body weight led to a decrease in insulin level and also had cytotoxic effect that was close to normal value.

**Keywords:** *Withania somnifera*, *Cnidium monnieri*, Diabetes, Obesity, Phytochemical analysis, Cytotoxicity

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### INTRODUCTION

Diabetes is characterized by improper metabolic symptoms of numerous conditions, known as long-term poor glycemic control, & results in impaired glucose, lipid, also protein metabolism due to reduced insulin production or insulin performance. At that time, important interventions in the development of science and technology as well as advanced medical centers were observed, protecting humans from the risks of many skin lesions and prolonging the life of the world. These interventions lead to cumulative death in the world, completely changing the epidemic and turning the epidemic aspect into an incurable disease. Over 70% of deaths throughout the world are expected to be caused

by widespread diseases. There are many complications associated with type 2 diabetes, which is one of the most prevalent non-contagious diseases (1).

A large portion of diabetes cases is caused by aging populations and obesity rising at an alarming rate. The global shift in lifestyle exacerbates harmful health practices, such as inactivity and poor nutrition. There is a growing epidemic of diabetes around the world, both in wealthy and poor nations. There are 8.8% of adults worldwide have diabetes at the moment. The prevalence of diabetes varies by region's demographics, including age, gender, and economic standing (2).

Human illnesses have been treated with plants for more than 60,000 years and crops are used in local programs to treat a variety of diseases. A total of 65% of the

worldwide population has been incorporated into the wellness system by the World Health Organization (WHO) (2003 rules) (see WHO Guidelines). The main role of plants as drugs is 1) bioactive elements are separated and purified and applied as medicines 2) Biologically active compounds are semi-synthesized using this compound to create known structures or new structures and generate less toxicity with high-activity compounds.

The major disadvantage of traditional drug treatment is immunosuppression. In modern medicine, plants are still at an early stage as a basis of immunomodulators. *W. somnifera* and *Cnidium monnieri* have shown immunomodulatory effects. A chemical compound osthol which is a derivative of coumarine, isolated from *C. monnieri* herbal exist in China. It has been used as a botanical coumarin compound as a glaze and energizer in China. It is effective against many disorders such as, allergy reactions, dermatophytes, many types of bacterial and fungal infections, osteoporotic, weakness, coldness, and skin-related disorders as a result of subcutaneous injection of *C. monnieri* (L.) CUSS alcoholic extract, the mating period was confirmed one day for 21 days of castrated mice. The consequences of this test show that this alcoholic extract containing osthol may possess activities of estrogen (3).

In medical field, *Withania somnifera* is acting as psycho pharmaceutical plant commonly utilized in Bharath and other countries. *Withania somnifera* is thought to be adaptogenic by many herbal doctors and scientists. It is helpful for curing long lasting complication which are due to nervous system or anxiety (4). Recent observations in our experimental dose surveys showed that *W. somnifera*. A relatively low daily dose of *Somnifera* oral extract increases stress resistance in laboratory rats. It shows that the effects of anti-anxiety or antidepressants is increasing in tense rodents with the number of treatment days (5). In this statement, the outcomes of an experiment resulting that test performed in harassed rats might be important to understand the pharmacology of low doses of *W. somnifera*. however, based on the current understanding of medicinal phytochemicals and phytopharmacology, we have briefly described the original hub and adaptation of biologically active ingredients of adaptogenic plants.

The purpose of this study was to check the qualitative & quantitative observations of *Withania somnifera* and *Cnidium monnieri* (CM). Animal models were used to explore the antidiabetic activity of selected plants compared with some standard allopathic drug.

## MATERIALS AND METHODS

### Animals handling and dosing

A group of animals of the same age and weight (120-160 g) were bought from the Department of

Physiology, Government College University, Faisalabad, and were bred for 21 days. Animals were divided into seven groups and were dosed in a way that the first group was normally controlled group and other groups were induced to diabetes by giving 100-200mg of and were given a high-fat diet. Afterward, the third group was treated with mevastatin and Glucophage (metformin HCL). The remaining groups were given high and low doses of *Withania somnifera* and *Cnidium monnieri* and compared their results.

### General methods for blood collection

To check the effect of plant extracts compared with allopathic drugs, animal trials using rats as an experimental model were performed. Rats were dosed with plant extract and protein diet at regular intervals and after three weeks blood sampling via heart puncture and blood test specimens were performed to test for biochemical analysis.

We collected blood specimens by the following methods:

- Anesthesia-free blood collection
- Saphenous vein (rat, mice, guinea pig)
- Dorsal pedal vein (rat, mice)

### Phytochemical Analysis

For phytochemical analysis tests for Tannins, Flavonoids, Glycosides, and triterpenoids were performed to evaluate the quantitative and qualitative assay of selected medicinal plant extracts (6). Ferric chloride test, lead acetate test, and saponin's test was used to check out the presence of tannins based on color indication as ferric chloride gave the violet color indication with tannins, and later two gave yellow-brown and white precipitate showing the presence of tannins. Shinoda's test and Alkaline reagent test were used to find out the presence of flavonoids based on color formation as Shinoda's test gave the reddish-purple indication and the alkaline reagent test gave the colorless result with flavonoids. Libermann-Bruchard Reaction, Legal's test, and Borntrager's test was used to check out the presence of glycosides based on color formation as the first one gave blue or red indicator, Legal's test gave the purple or red while the last one gave the purple indication showing the presence of glycosides (7). Libermann Burchard test, Noller test, and Salwoski test were used to check out the presence of triterpenoids based on color formation as the first one gave the reddish violet color, red color was. developed with Nobler's test and the last one gave blue green indication showing the presence of triterpenoids (8).

### Biochemical Analysis

The complete blood count (CBC) was conducted to evaluate the blood-circulating (9) cells including White blood cell count (WBC or leukocyte count), WBC differential count Red blood cell count (RBC or erythrocyte count), Haematocrit (Hct), Haemoglobin (Hbg) Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) Mean corpuscular haemoglobin concentration(MCHC), Red cell distribution width (RDW), Platelet count Mean Platelet Volume (MPV). In order to measure the amount of glucose or sugar in a person's blood, random glucose tests are used (10). Tests that were used to check out either the liver was performing proper functions or not included Alanine Transaminase (ALT) test Aspartate Transaminase (AST) test, Alkaline phosphatase, (ALP) Test and Bilirubin test. A lipid profile test was performed to check out the total cholesterol, LDL, HDL, and triglycerides level in blood (11).

### Final sampling

After six weeks, the final sampling was performed. Sampling was performed by providing animals anesthesia. For this purpose, chloroform was used. The animals were then sacrificed for further assessment. Liver and pancreas were detached for biopsy

## RESULT AND DISCUSSION

**Table.01** Qualitative analysis of *Withania somnifera* and *Cnidium monnieri*

Plants/ Phytochemicals	<i>Withania somnifera</i>	<i>Cnidium monnieri</i>
Alkaloids	+	-
Flavonoids	+	+
Tannins	+	-
Saponins	+	-
Glycosides	+	+
Steroids	+	+
Triterpenoids	+	+

**Table 02** Quantitative analysis of *Withania somnifera* and *Cnidium monnieri*

Plants/ Phytochemicals	<i>Withania somnifera</i>	<i>Cnidium monnieri</i>
TPC (mg GAE/g dry plants material)	179.51 ± 5.63	291.99 ± 8.43
TFC (µg CE/g dry plants material)	35.76 ± 0.53	77.1 ± 0.90
DPPH Scavenging activity (%)	47.41 ± 3.13	36 ± 4.16

Phytochemical analysis of *Withania somnifera* and *Cnidium monnieri* discussed in table 1 (qualitative analysis) and in table 2 (quantitative analysis). Phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids were present in *Withania somnifera*. Phytochemicals such as falvonoids, glycosides, steroids, triterpenoids were present in *Cnidium monnieri*.

Quantitative analysis of *Withania somnifera* and *Cnidium monnieri* discussed in following table. Analysis results showed in basic units of TPC, TFC, DPPH activity. *Withania somnifera* or Ashwagandha is widely used as a folk medicine to treat multiple illnesses. It is a natural origin of withanolides (steroidal lactones), a phytochemical that is used as a component in many formulations prescribed for a multitude of illnesses. It functions as a sedative, diuretic, and anti-inflammatory agent usually used to increase energy and strength and acts as an adaptogen that functions as a powerful immune stimulator and anti-stress agent.

Medicinal plants retain the health & vitality of people and also heal illness without causing poisoning. The accessible scientific data favor the hypothesis that Ashwagandha is a powerful regenerative tonic due to its various pharmacological activities such as anti-stress, neuroprotective, anti-tumor arthritis, analgesic, anti-inflammatory, etc.

**Table 03:** A Lipid profile Assay of Animal models for different groups. All the results show P value as follows P<0.05.

Groups	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)
Normal	41.03±0.039	10.24±12.49	51.72±8.4	57.21±8.8
Negative control (Intoxicated)	101.5±9.7	18.33±8.4	70.00±11.6	81.79±6.3
Standard Drug	40.02±0.02	11.21±10.47	50.71±9.3	58.31±9.8
WS (Low)	43.8±6.84	8.17±6.64	17.27±5.33	40.6±5.9
WS (High)	51.6±7.51	8.63±11.92	75.17±12.31	52.06±8.2
CM (Low)	44.5±6.89	9.24±11.93	28.33±10.11	44.63±7.6
CM (High)	41.5±6.03	7.91±9.32	26.95±8.2	40.00±5.6

It is helpful for various kinds of illnesses such as Parkinson's disease, dementia, memory loss, stress-induced illnesses, malignancies, and others. *Withania somnifera* root medicine has a significant position in the therapy of rheumatic pain, swelling of the joints, nervous illnesses, and epilepsy. Dry roots are used as a tonic for hiccups, colds, coughs, sexual illnesses, sedatives, senile debility, etc. Leaves are used for carbuncles, inflammation and swelling. Leaf juice is helpful for conjunctivitis. It has anti-inflammatory, anti-tumor, anti-stress, anti-oxidant, mind-boosting, immune-enhancing, and rejuvenating characteristics (12). The current research shows the existence of phenolic compounds, flavonoids and antioxidants in WSREt and WSLet and also offers evidence to promote the restoration of alloxan-induced diabetic harm in rats (13).

### Lipid Profiles

Normal range of Cholesterol, HDL, LDL, Triglycerides were as 41.03±0.039, 10.24±12.49, 51.72±8.4 and 57.21±8.8 respectively, these values were closed to their standard values. When these rats were intoxicated their values were increased in the range of 101.5±9.7, 18.33±8.4, 70.00±11.6 and 81.79±6.3 respectively as compared with the results (14) our study is comparative.

But as a treatment when plants *Withania somnifera* and *Cnidium monnieri* were given the results were obtained as follows. Experiments performed with reference to (15), when *Withania somnifera* was used in low concentration Intoxicated rats showed relatively decreased in their Lipid profiles value such as cholesterol value 101.5±9.7 decreased to 43.8±6.84, HDL value 18.33±8.4 decreased to 8.17±6.64, LDL value decreased 70.00±11.6 to 17.27±5.33 and Triglycerides value 81.79±6.3 decreased to 40.6±5.91. When High concentration of *Withania somnifera* extracts was given to diseased rats remarkable changes in their values was observed such as cholesterol level 41.03±0.039 had minutely increased to 51.6±7.51, HDL value 18.33±8.4 decreased to 8.63±11.92 which was

closed to normal value, LDL value 70.00±11.6 increased to 75.17±12.31 when a high dose of *Withania somnifera* was given to rats. Diseased rats had shown great decrease in Triglycerides value when extracts of high dose of *Withania somnifera* were given to them, their value decreased from 81.79±6.3 to 52.06±8.2 but this change was not as remarkable as when high concentration of *Withania somnifera* extracts were used in controlling Triglycerides value. When second plant *Cnidium monnieri* (CM) was given to treat the diseased rats, significant decrease in intoxicated rats' values were observed as discussed in the above table. When low dose of CM plants was used cholesterol, value decreased from 101.5±9.7 44.5±6.89, HDL value came in range of 9.24±11.93 from 18.33±8.4, LDL value 70.00±11.6 decreased to 28.33±10.11 and Triglycerides value 81.79±6.3 decreased to 44.63±7.6. But when high dose of CM plant extracts was injected to intoxicated rats, greater differences were observed. Cholesterol, HDL, LDL, Triglycerides values 101.5±9.7, 18.33±8.4, 70.00±11.6 and 81.79±6.3 were decreased to 41.5±6.03, 7.91±9.32, 26.95±8.2, 40.00±5.6 respectively. High dose of CM plants had positive effects in controlling Cholesterol and HDL values in intoxicated rats as compared with the results (16). All the results show P value as follows P<0.05.

### Hormonal Test

Normal range of Insulin, T3, T4, THS were 1.63±1.93, 1.63±1.93, 10.37±1.48 and 0.03±0.055 respectively, these values were closed to the values when standard drug was used as in the range of 1.53±1.87, 0.54±0.05, 10.36±1.39 and 0.03±0.049. When rats were intoxicated their normal range, values of Insulin T3, T4, TSH were increased in range of 2.18±1.62, 1.67±0.115, 16.57±0.51 and 0.66±0.140 respectively.

After intoxication, these rats were treated with our two medicinal plants *Withania somnifera* and *Cnidium monnieri* (CM) in high and low dose trials planned with re comparative study of (17).

**Table 04:** Hormonal Assays of Animal models for different groups. All the results show P value as follows P<0.05.

Groups	Insulin (mg/dL)	T3 ( $\mu$ g/dL)	T4 (ng/dL)	THS (m U/L)
Normal	1.63 $\pm$ 1.93	1.63 $\pm$ 1.93	10.37 $\pm$ 1.48	0.03 $\pm$ 0.055
Negative control (Intoxicated)	2.18 $\pm$ 1.62	1.67 $\pm$ 0.115	16.57 $\pm$ 0.51	0.66 $\pm$ 0.140
Standard Drug	1.53 $\pm$ 1.87	0.54 $\pm$ 0.05	10.36 $\pm$ 1.39	0.03 $\pm$ 0.049
WS (Low)	2.34 $\pm$ 0.61	0.38 $\pm$ 0.068	7.33 $\pm$ 0.711	0.43 $\pm$ 0.318
WS (High)	1.59 $\pm$ 1.46	0.50 $\pm$ 0.03	11.10 $\pm$ 1.05	0.05 $\pm$ 0.004
CM (Low)	1.50 $\pm$ 1.42	0.38 $\pm$ 0.05	9.27 $\pm$ 0.153	0.34 $\pm$ 0.25
CM (High)	1.40 $\pm$ 1.47	0.31 $\pm$ 0.03	8.03 $\pm$ 0.153	0.23 $\pm$ 0.015

When the low dose of *Withania somnifera* were given to rats, insulin concentration was not controlled in the normal range, but T3 value decreased from 1.67 $\pm$ 0.115 to 0.38 $\pm$ 0.068 that was less than normal range of T3 value, T4 value 16.57 $\pm$ 0.51 decreased to 7.33 $\pm$ 0.711 which was also lower than normal value and THS value 0.66 $\pm$ 0.140 decreased to 0.43 $\pm$ 0.318. When high dose of *Withania somnifera* were given insulin value 2.18 $\pm$ 1.62 decreased to 1.59 $\pm$ 1.46 which was very close to normal value of insulin, T3 value 1.67 $\pm$ 0.115 decreased to 0.50 $\pm$ 0.03 which was less than the normal range, T4 value 16.57 $\pm$ 0.51 decreased to 11.10 $\pm$ 1.05, THS value 0.66 $\pm$ 0.140 decreased to 0.05 $\pm$ 0.004 which was close to normal range value of THS.

When the low dose of *Cnidium monnieri* (CM) plant extracts was given to intoxicated rats, remarkable changes were observed in rats Hormonal profiles as discussed in the above table. Insulin value 2.18 $\pm$ 1.62 decreased to 1.50 $\pm$ 1.42, T3 value 1.67 $\pm$ 0.115 decreased to 0.38 $\pm$ 0.05, T4 value 16.57 $\pm$ 0.51 decreased to 9.27 $\pm$ 0.153 and THS value 0.66 $\pm$ 0.140 decreased to 0.34 $\pm$ 0.25, when a high dose of CM plant extract was given to intoxicated rats, and results were close to their normal ranges. After that High dose of CM plant extracts were given to rats and changes were observed as follows.

Insulin value 2.18 $\pm$ 1.62 decreased to 1.40 $\pm$ 1.47, T3 value 1.67 $\pm$ 0.115 decreased to 0.31 $\pm$ 0.03, T4 value 16.57 $\pm$ 0.51 decreased to 8.03 $\pm$ 0.153 and THS value 0.66 $\pm$ 0.140 decreased to 0.23 $\pm$ 0.015. High dose of both plants had positive effects in controlling the hormones in their normal ranges (18). All the results show P value as follows P<0.05.

#### Cytotoxic activities and hepatoprotective

Normal range of Urea, Creatinine, Blood sugar rate and Uric acid were observed as 28.66 $\pm$ 40.49, 1.03 $\pm$ 1.70, 0.59 $\pm$ 0.06 and 2.03 $\pm$ 1.58 respectively, these values were close to the values

when the standard drug was used as in the range of 27.56 $\pm$  39.40, 1.02 $\pm$ 1.69, 0.58 $\pm$ 0.06 and 2.02 $\pm$ 1.59. When rats were intoxicated their normal range, values of Urea, Creatinine, Blood sugar rate, and Uric acid were increased in the range of 53.39 $\pm$ 72.12, 1.14 $\pm$ 1.61, 1.67 $\pm$ 0.11 and 5.04 $\pm$ 3.87 respectively. After intoxication, these rats were treated with our two medicinal plants *Withania somnifera* and *Cnidium monnieri* (CM) in high and low dose trials (19).

When low dose of *Withania somnifera* were given to rats, Urea concentration 53.39 $\pm$ 72.12 decreased to 21.73 $\pm$ 33.73 and this value was less than normal value, creatinine value 1.14 $\pm$ 1.61 decreased to 1.03 $\pm$ 1.71 that was same as the normal range of creatinine value, Blood sugar rate value 1.67 $\pm$ 0.11 decreased to 0.38 $\pm$ 0.06 which was quite close to normal value and Uric acid concentration 5.04 $\pm$ 3.87 decreased to 2.99 $\pm$ 1.49 that was also equal to normal range value. When high dose of *Withania somnifera* was given Urea value 53.39 $\pm$ 72.12 decreased to 30.12 $\pm$ 44.38 which was high than normal value of urea, creatinine value 1.14 $\pm$ 1.61 decreased to 1.04 $\pm$ 1.70 which was close to normal range, Blood sugar rate value 1.67 $\pm$ 0.11 decreased to 0.50 $\pm$ 0.03, Uric acid value 5.04 $\pm$ 3.87 decreased to 3.68 $\pm$ 1.57 which was high than normal range value of Uric acid. When low dose of *Cnidium monnieri* (CM) plant extracts were given to intoxicated rats, remarkable changes were observed in rats' Cytotoxic activities and hepatoprotective activity profile as discussed in the above table. Urea concentration 53.39 $\pm$ 72.12 decreased to 25.85 $\pm$ 37.38, and creatinine value 1.14 $\pm$ 1.61 decreased to 1.05 $\pm$ 1.68. Blood sugar rate value 1.67 $\pm$ 0.11 decreased to 0.38 $\pm$ 0.04 and Uric acid value decreased to 2.01 $\pm$ 1.68 which was close to the normal range value of uric acid.

After that High dose of CM plant extracts was given to rats and changes were observed as follows Urea concentration value 53.39 $\pm$ 72.12 decreased to 23.61 $\pm$ 32.71, creatinine value 1.14 $\pm$ 1.61 decreased to

**Table 05:** Renal failure analysis of blood samples of animal models. All the results show P value as follows P<0.05

Groups	Urea (mg/dL)	Creatinine (mg/dL)	Blood sugar rate (mmol/L)	Uric acid (mg/dL)
Normal	28.66±40.49	1.03±1.70	0.59±0.06	2.03±1.58
Negative control (Intoxicated)	53.39±72.12	1.14±1.61	1.67±0.11	5.04±3.87
Standard Drug	27.56± 39.40	1.02±1.69	0.58±0.06	2.02±1.59
WS (Low)	21.73±33.73	1.03±1.71	0.38±0.06	2.99±1.49
WS (High)	30.12±44.38	1.04±1.70	0.50±0.03	3.68±1.57
CM (Low)	25.85±37.38	1.05±1.68	0.38±0.04	2.01±1.68
CM (High)	23.61±32.71	1.04±1.69	0.31±0.03	2.40±1.31

1.04±1.69, Blood sugar rate value 1.67±0.11 decreased to 0.31±0.03 and Uric acid value 5.04±3.87 decreased to 2.40±1.31. High and Low doses of both plants had quite positive effects in controlling the Urea, Creatinine, Blood sugar rate, and Uric acid in their normal ranges. All the results show the P value as follows P<0.05.

## CONCLUSION

Aging populations in the world and a tremendous increase in obesity played an important role in the development of diabetes. The incidence of diabetes depends on the age, gender, and socioeconomic position of each geographic zone. The prevalence of diabetes is 22% in developing countries and 75 to 79 years in developing countries, 19% in the 60- to 70-year-old group in developing countries and underdeveloped countries, and 8% in the 55-64-year-old group in underdeveloped countries (14). The macroscopic vascular complexity of diabetes plays an important role in the increase of arteriosclerosis, as well as in the acceleration of morbidity and mortality and the aggravation of the incidence. Researchers could not find methods of interaction of both diabetes and atherosclerosis. Oxidation due to highly reactive oxygen species is considered the main cause of insulin resistance, vascular complexity and type 2 diabetes mellitus. Requirements include animal, anesthetic agent, surgical blade, small glass rods, surgical scissor, 21 to 25 G needle with 1 to 5 ml syringe and blood sample collection tube (15). The animals were dosed with plant extract and protein diet. Then after three weeks re-sampling was performed. Blood samples were gathered by heart puncture and blood test specimens were used. Animals were dosed with plant extract and protein diet. After three weeks; re-sampling was conducted. Blood samples were collected by cardiac puncture and blood samples were used. The complete blood count (CBC) is a blood-circulating cell exam. CBC is typically performed using an automated tool that measures multiple parameters, including the number of cells in the blood sample of a person.

Phytochemicals such as flavonoids, glycosides, steroids, and triterpenoids were present in *Cnidium monnieri*. The current research shows the existence of phenolic compounds, flavonoids and antioxidants (20) in WSREt and WSLEt and also offers evidence to promote the restoration of alloxan-induced diabetic harm in rats. Impaired glucose tolerance and insulin resistance, as well as being an efficient means of regulating glucose levels.

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