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In vivo exploration of *Withania somnifera* and *Cnidium monnieri* in experiment animals against diabetic disorder

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Key words: Diabetes mellitus, *Withania somnifera, Cnidium monneri*, Hyperglycemia, Biochemical parameters.

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Abstract

Diabetes is the most important deadly metabolic disorder which can disturb nearly every organ system in the body. An increase in sugar levels is one of the characteristics of diabetes mellitus, which leads to a serious impact on the heart, blood vessels, eye and kidneys from time to time. To cure the diabetes we use herbal plants like *Cnidium monnieri* and *Withania somnifera* which have immunomodulatory effects and phytochemical analysis confirms the presences of flavonoids, alkaloids, steroids and glycosides. These phytochemicals have have antioxidents anti-diabetic, anti-stress, anti-arthritis, immune enhancing and anti-inflammatory activities which prove when *in-vivo* exploration of these plants extract in diabetic mice to check thr therapeutic potential and the results of different biochemical parameters encourage the research purpose and declear that these plants have anti-diabetic activity. In the future, these herbal plants will extensively be used in medicines of certain diseases.

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Introduction

Diabetes comprises the inappropriate metabolic symptoms of many illnesses, called long-term poor glycemic control, and causes poor glucose, fat, and protein metabolism due to decreased insulin output or decreased insulin performance. About 70% of deaths in the world are expected to be caused by outspread diseases. Type 2 diabetes is one of the most prevalent non-contagious diseases that cause serious complications (Anne *et al.,* 2018). The world's elderly groups and the huge rise in obesity played a significant part in diabetes build-up. Global lifestyle changes contribute to damaging health habits, including the absence of fitness and bad quality of nutrition. In high-income countries and low-income countries as well, diabetes is a quickly expanding issue. The global Diabetes incidence is currently 8.8% (Weisman and Rodebaugh, 2018). The incidence of diabetes depends on the age, gender and socioeconomic position of each geographic zone. Diabetes incidence is 22% in the developed nations and 75% to 79% in the emerging nations, 19% in the 60-70-year-olds group and 8% in the 55-64-year-olds group in the emerging nations (Shaw *et al.,* 2010). At the end of 2017, 424.9 million individuals are expected to have diabetes in 2045, at 626.6 million, according to the International Diabetes Federation (IDF). Besides, 50 percent of diabetically ill and 3/4 of their lives in low income or middle-income nations do not know the disease. (Cosansu *et al.,* 2018).

High blood glucose levels cause microvascular (neuropathy, retinopathy, nephropathy) and macrovascular complications (stroke, coronary artery disease). Diabetes is growing worldwide and clinical equipment is also increasing for main and secondary complications. So, doctors must find the interaction of diabetes and vascular diseases (Fowler, 2008; Heydari *et al.,* 2010; Pedersen *et al.,* 2018). It is essential for the treatment of diabetes, it should be identified at Initial stages diagnosis, so hazards of high blood glucose can be decreased (Parasad *et al.,* 2012). The following symptoms help in the identification of diabetes such as the production of large urine, excessive thirstiness, polyphagia, infections and mysterious weight loss (Ahmad *et al.,* 2018). By measuring the glucose level in blood can approve the symptoms. In about 50% of cases, symptoms have not appeared, but glucose level during fasting is 7 mmol / L or more for 2 hours, glucose tolerance is 11.1 mmol / L or more, 6.5% is glycated hemoglobin (AIC) or can expect the progress of retinopathy. These parameters are very useful for the identification of diabetes among the human population (Cai *et al.,* 2014). If it occurs slowly or if it is not diagnosed, it is a sign of complications of diabetes leading to a loss of quality of life, and economic disasters and premature deaths seriously affect the healthcare system. Most of these problems are associated with most side effects such as loss of legs, vision problems, complications of kidney, neuropathy, loss of tops, and cardiovascular complications (Attila *et al* ., 2011).

Medicinal plants are used for the curing of human illnesses over 60,000 years old (Solecki, 1975) and crops for the therapy of various illnesses can be used. Many plants like *Cnidiummonnieri Withaniasomnifera* show immunomodulatory effects. They are effective to treat the number of disorders. *Withania somnifera* belongs to family Solanaceaceis also known in the subcontinent as Asgawandha, where it is used as a traditional drug (Chandran *et al.,* 2017; Gupta &Chhikara, 2018). It is used to treat diabetes mellitus and has investigated its antidiabetic activity with various animal models and diabetes patients. Leaves and root extract of Asgawandha increased the capacity of myotube and adipocyte to absorb glucose (Jonathan *et al.,* 2015). *Withania somnifera* is ethnomedicine in the Ayurvedic system of medicine (Bhattacharya *et al.,* 2003). It was classified by Ayurvedic physicians as “rasayana”, a kind of drug derived from plants which boost up the physio-psychological health and provide immunity against certain disease like nervous coordination and chemical coordination, heart disease, anticoagulant, tumor repressor, adaptogenic, immunostimulatory, antioxidant and anti-rheumatic actions (Mishra *et al.,* 2000). It also has a good effect on estrogen (Kaur *et al.,* 2017). aOn the other hand in the local population,

for a long time, *Cnidiummonnieri* (Umbelliferae) is utilized as a diet and for therapeutics.m*C. monnieri* has an immunostimulatory effect on macrophages (Mall*et al.,* 2016). Osthole is an agent isolated from Cnidiummonnieri (L.) is very useful for the treatment of various diseases, including metabolic syndromes. It is also effective against diabetes as it regulates glucose metabolism by activation of PPARalpha and PPARgamma (Peroxisome proliferator-activated receptor). According to studies, Phenol glycosides present in *C. monnieri* have immunostimulatory effects. (Kin *et al.,* 2013).

So, the main purpose of the study is to find the effective treatment of diabetes with the help of phytochemicals including flavonoids, alkaloids, steroids, glycosides and many other bioactive compounds that are extracted and purified from these plants. We also check the therapeutic potential of these phytochemicals against other illness that occurred in diabetic patients like heart diseses, liver and kidneys imbalance. Moreover we focus to find the treatment of diabete with cost effectiveness.

Materials and methods

*Phytochemical analysis*Test for Tannins (Ferric chloride test): Added to 1-2 ml of extract and a few drops of 5 percent FeCl3 aqueous solution. The development of a violet color demonstrates the presence of tannins.

Test for Saponins: The sample was diluted with 20 ml distilled water and put in a graduated cylinder for 15 minutes. The formation of a 1 cm coating of foam shows the presence of Saponins.

Test for Flavonoids (Shinoda's test): In a test tube containing 0.5 ml of oil, 5-10 drops of diluted HCl and a small piece of ZnCl or magnesium were introduced and the solution was chi for a few minutes. Reddish purple color has been produced in the presence of flavonoids.

Test for Glycosides (Legal's test): Add 1 ml of pyridine and a few drops of sodium nitroprusside alternative to hydrolysate, and then alkaline with sodium hydroxide solution was created. The appearance of purple to red shows the presence of glycosides.

Test for Triterpenoids (LibermannBurchard test):10 mg of the sample was mixed in 1 ml of chloroform and 1 ml of acetic anhydride was placed in 2 ml of Conc. H2SO4, man. The development of reddish-violet color indicates the presence of triterpenoids.

Test for Phytosterol (Salkowski test): The residue has been dissolved in a few drops of diluted acetic acid and a couple of drops of Conc have been added to 3 ml of acetic anhydroid. H2SO4.-H2SO4. The blue-green appearance indicates the phytosterol existence.

Study subject

Experimental mice of the order Rodentiawere use as experiment purposes and bought from the Department of Physiology, Government College University, Faisalabad. These animals have been breading for 21 days. Animals of the same age group and the same weight (120-160 g) were breaded and given high lipids and fats diet.

Study design

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. Blood sampling was done at different levels. For this purpose, Initial sampling is normal sampling and taken blood samples through the tail vein. This is done at the initial stage to check the normal metabolic activities. After that Streptozotocin is given to the rats which leads to hypoinsulinemia and hyperglycemia condition and induced diabetes mellitus.Then, to check the therapeutic potential of phytochemicals against diabetes, 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.

Biochemical tests

To check the *in vivo* activity of plant extract some

biochemical tests were performed. RBS (random glucose test is one technique of measuring the quantity of glucose or sugar circulating in a person's blood), blood insulin, blood Urea Nitrogen (BUN), blood creatinine (if urea and creatinine level increase in the blood it reduces kidney function), blood cholesterol test(that measures the amount of each type of cholesterol), HDL rate, LDL rate (too much LDL cholesterol in your blood may put you at risk for heart disease), SGPT (to measure the liver damage), blood triglycerides (a lipid profile is a test that measures the level of fats in the blood) and blood uric acid test (high level of uric acid causes painful joints). All these tests must be performed because the chance of heart problem, stroke and long-lasting kidney complications is increased in the case of diabetes mellitus.

Statistical analysis

Statistical analysis was performed. Expressive statistics such as mean, standard deviation and standard error values were calculated for all the parameters. Interval wise evaluation was approved for all the parameters by using repetitive measures with nine doses.

Investigated the discrepancy to comprehend the therapeutic potential of *Withania somnifera and Cnidium monnieri* plant extract. Further graphical representations were made by using bar graphs.

Results

Phytochemical analysis

Phytochemical analysis of *Withaniasomnifera*and *Cnidiummonnieri* discussed in Table 1 (qualitative analysis) and Table 2 (quantitative analysis).

Phytochemicals such as flavonoids, tannins, saponins, glycosides, steroids, triterpenoids were present in *Withania somnifera*. Phytochemicals such as falvonoids, glycosides, steroids, triterpenoids were present in *Cnidium monnieri*.

Table 1. Qualitative analysis of *Withania somnifera and Cnidium monnieri.*

Plants/ Phytochemicals	*Withania somnifera*	*Cnidium monnieri*
Flavonoids	+	+
Tannins	+	-
Saponins	+	-
Glycosides	+	+
Steroids	+	+
Triterpenoids	+	+

Table 2. Quantitative analysis of *Withania somnifera and Cnidium monnieri.*

Plants/ Phytochemicals	*Withaniasomnifera*	*Cnidiummonnieri*
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

*GAE/g (gallic acid equivalent in 1gram of material), µg CE/g (micro-gram cathechin equivalents per gram dry material). These are the basic unit of total phenolic and flavonoids contents respectively.

*DPPH Scavenging activity (DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. In the DPPH assay, violet color DPPH solution is reduced to yellow colored product, diphenylpicryl hydrazine, by the addition of the extract in a concentration dependent manner).

Quantitative analysis of withaniasomnifera and cnidiummonnieri discussed in following table. Analysis results showed in basic units of TPC (total phenolic contents), TFC (total flavonoids contents), DPPH activity (DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer.

Statistical and graphical analysis of biochemical parameters

Twenty-four mice of the order Rodentia were enrolled in this study which fulfilling the inclusion criteria. These animals have been breading for 21 days. Animals of the same age group and the same weight (120-160 g) were breaded and given high lipids and

fats diet. The plant extract was given in nine different doses within three weeks and blood analysis was carried out. The gradual recovery in all mice was observed. During the first three doses, all biochemical parameters show elevation from normal values. But further doses gradually decrease the values till we find normal values required for the proper functioning of β- cells of pancreas, kidneys, liver and heart.

Table 3. Statistical analysis of blood sugar rate.

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean
D1	3	0.5967	0.06429	0.03712
D2	3	1.667	0.1155	0.06667
D3	3	1.170	0.1473	0.08505
D4	3	0.5900	0.1153	0.06658
D5	3	0.4567	0.05508	0.03180
D6	3	0.3833	0.06807	0.03930
D7	3	0.5033	0.03055	0.01764
D8	3	0.3833	0.04933	0.02848
D9	3	0.3100	0.02646	0.01528

Table 4. Statistical analysis of insulin rate in mice.

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean
D1	3	1.632	1.934	1.368
D2	3	2.185	1.621	0.9360
D3	3	1.940	1.557	0.8987
D4	3	1.595	1.424	0.8221
D5	3	1.500	1.453	0.8386
D6	3	2.342	0.6195	0.3577
D7	3	1.589	1.462	0.8441
D8	3	1.500	1.428	0.8246
D9	3	1.403	1.474	0.8510

Descriptive analysis such as mean, standard deviation and standard error values were calculated and found to be noteworthy for all parameters in the following tables (3-12) Further, to represent the effect of the *Withania somnifera* and *Cnidium monnieri*, the period wise evaluation was performed by using repetitive measures Analysis of Variance.

Table 5. Statistical analysis of urea rate in mice.

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean
D1	3	28.657	40.489	23.377
D2	3	53.389	72.118	41.637
D3	3	57.613	90.718	52.376
D4	3	28.013	39.598	22.862
D5	3	27.469	37.246	21.504
D6	3	21.731	33.727	19.472
D7	3	30.120	44.378	25.621
D8	3	25.856	37.386	21.585
D9	3	23.613	32.714	18.887

Table 6. Statistical analysis of creatinine in mice.

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean
D1	3	1.034	1.703	0.9835
D2	3	1.144	1.617	0.9333
D3	3	1.188	1.578	0.9113
D4	3	1.297	1.476	0.8522
D5	3	1.033	1.704	0.9836
D6	3	1.026	1.710	0.9873
D7	3	1.037	1.701	0.9820
D8	3	1.055	1.685	0.9728
D9	3	1.039	1.699	0.9807

Using this, deviations in the parameter values were observed for RBS, blood insulin, blood Urea Nitrogen (BUN), blood creatinine, blood cholesterol test, HDL rate, LDL rate, SGPT, blood triglycerides and blood uric acid test. Dose wise significance has also been indicated with the help bar graphs (1-10). We observed that the values for these parameters after the fourth dose are found to be statistically significant as compared to the first three doses.

Similarly, a gradual decrease in the blood glucose level was also observed in all diabetic mice.

Table 7. Statistical analysis of cholesterol rate in mice.

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean
D1	2	3.028	0.03893	0.02753
D2	3	59.484	97.154	56.092
D3	3	59.789	96.606	55.775
D4	3	50.227	74.966	43.282
D5	3	51.394	44.106	25.465
D6	3	43.725	68.377	39.478
D7	3	51.583	75.032	43.320
D8	3	44.519	68.855	39.754
D9	3	41.512	60.296	34.812

Table 8. Statistical analysis of HDL rate in mice.

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean
D1	3	10.241	12.493	7.213
D2	3	18.333	24.846	14.345
D3	3	18.422	25.057	14.466
D4	3	9.911	13.083	7.554
D5	3	9.061	10.919	6.304
D6	3	8.175	6.643	3.836
D7	3	8.637	11.923	6.884
D8	3	9.244	11.930	6.888
D9	3	7.916	9.322	5.382

This means that on using plant extract, blood insulin increases to a safe level and start to utilize extra blood glucose. As well as plants extract have several phytochemicals which helped to balance the related dysfunctions of liver, kidneys and heart. After three weeks of administration of plant extracts, no adverse reactions were observed indicating the non-toxic nature of plant extract.

Table 9. Statistical analysis of LDL rate in mice.

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean
D1	3	51.728	84.818	48.970
D2	3	70.000	116.05	67.000
D3	3	67.415	108.77	62.799
D4	3	28.607	45.960	26.535
D5	3	21.829	34.224	19.759
D6	3	17.274	26.337	15.206
D7	3	75.175	122.54	70.750
D8	3	28.333	42.158	24.340
D9	3	26.947	39.326	22.705

Table 10. Statistical analysis of SPGT rate in mice.

Group	Number of points	mean	Standard Deviation	Standard Error of mean
D1	3	14.287	20.838	12.031
D2	3	30.637	49.981	28.857
D3	3	24.065	37.768	21.805
D4	3	18.960	27.459	15.853
D5	3	17.818	24.987	14.426
D6	3	11.548	15.407	8.895
D7	3	19.166	26.998	15.588
D8	3	15.667	23.692	13.679
D9	3	12.967	13.760	7.944

In the present study, we found the therapeutic potential of *Withania somnifera* and *Cnidium monnieri* extract in the treatment of diabetes and related dysfunctioning.

Table 11. Statistical analysis of triglycerides rate in mice.

Groups	Number of points	Mean	Standard Deviation	Standard Error of Mean
D1	3	57.210	88.780	51.257
D2	3	81.792	125.35	72.369
D3	3	77.928	124.23	71.727
D4	3	49.825	77.829	44.935
D5	3	44.089	69.501	40.126
D6	3	40.580	59.908	34.588
D7	3	52.064	82.518	47.642
D8	3	44.629	67.070	38.723
D9	3	40.033	54.005	31.180

Table 12. Statistical analysis of Uric acid rate in mice.

Groups	Number of points	Mean	Standard Deviation	Standard Error of Mean
D1	3	2.033	1.589	0.9171
D2	3	5.043	3.871	2.235
D3	3	4.338	3.979	2.297
D4	3	3.051	2.491	1.438
D5	3	2.525	1.696	0.9791
D6	3	2.996	1.499	0.8656
D7	3	3.681	1.567	0.9049
D8	3	2.010	1.681	0.9703
D9	3	2.400	1.308	0.7550

Discussion

Withania somnifera or ashwagandha and *Cnidium monnieri* are widely used as a folk medicine to treat multiple illnesses. Our research decleared that there are number of phytochemicals like phenols, flavonoids glycosides and steroids are present in extracts of these plants which are affective against diabetes.

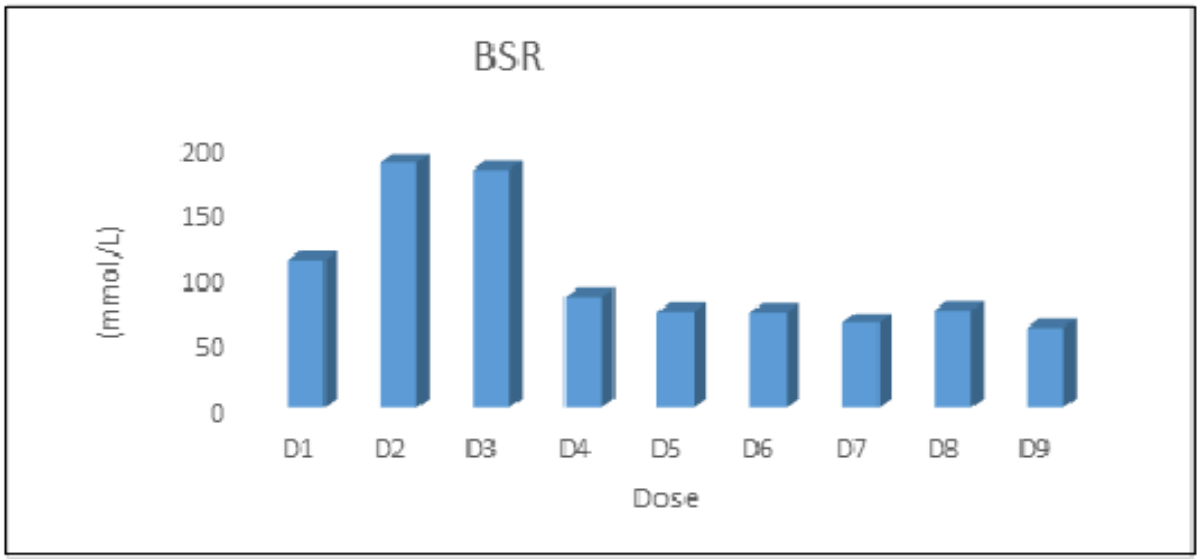

Fig. 1. Graphical representation of BSR in mice.

Moreover it conforms that these phytochemicals have antioxidents anti-diabetic, anti-stress, anti-arthritis, immune enhancing and anti-inflammatory activity which exibit protection from oxidative damages and reactive oxygen species. So that the diabetic patients get cured from the related diseases like heart disease, mucular degeneration kidneys and liver imbalance and nervous degeneration.

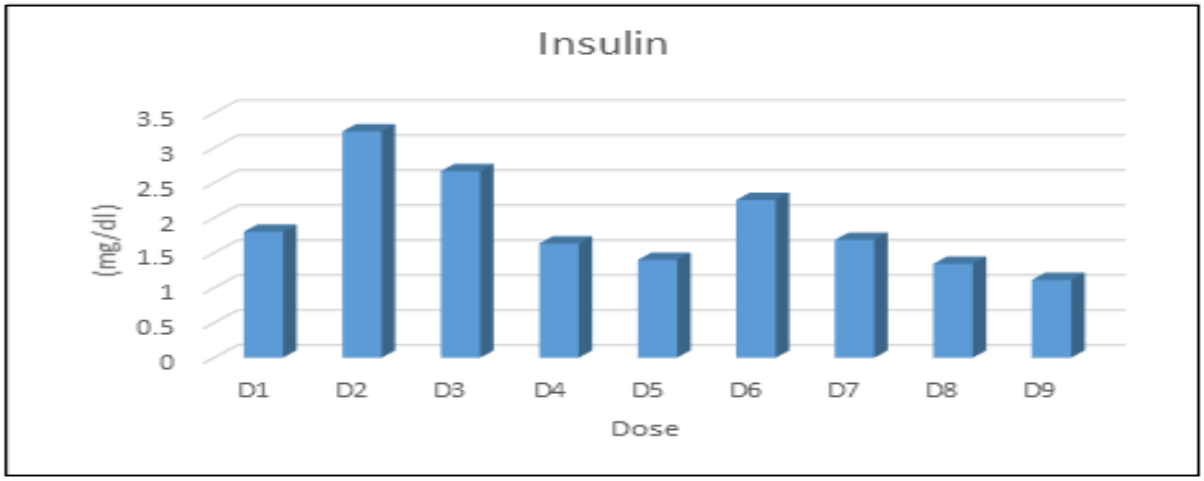

Fig. 2. Graphical representation of insulin in mice.

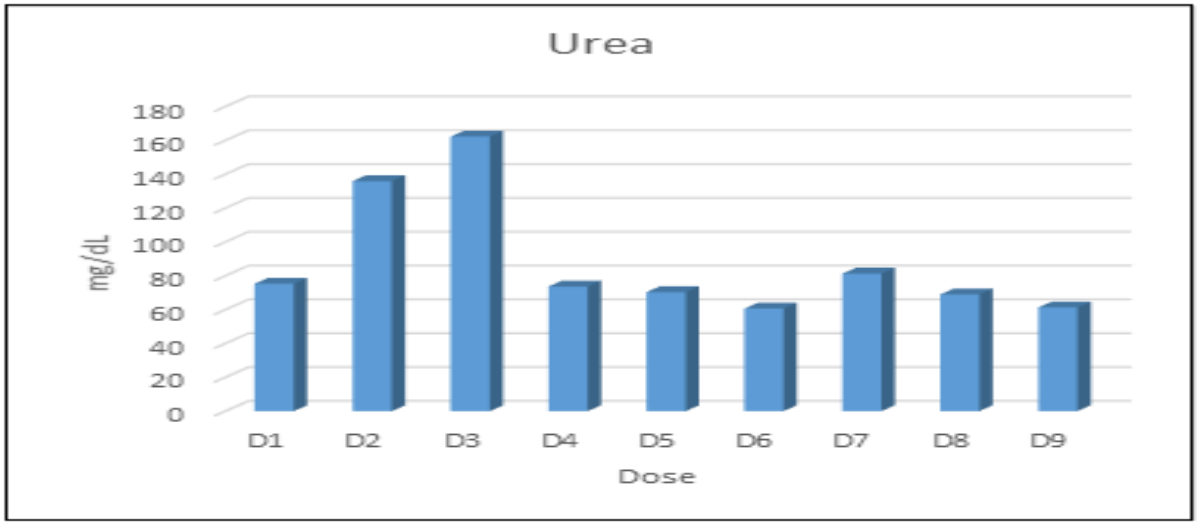

Fig. 3. Graphical analysis of urea in mice.

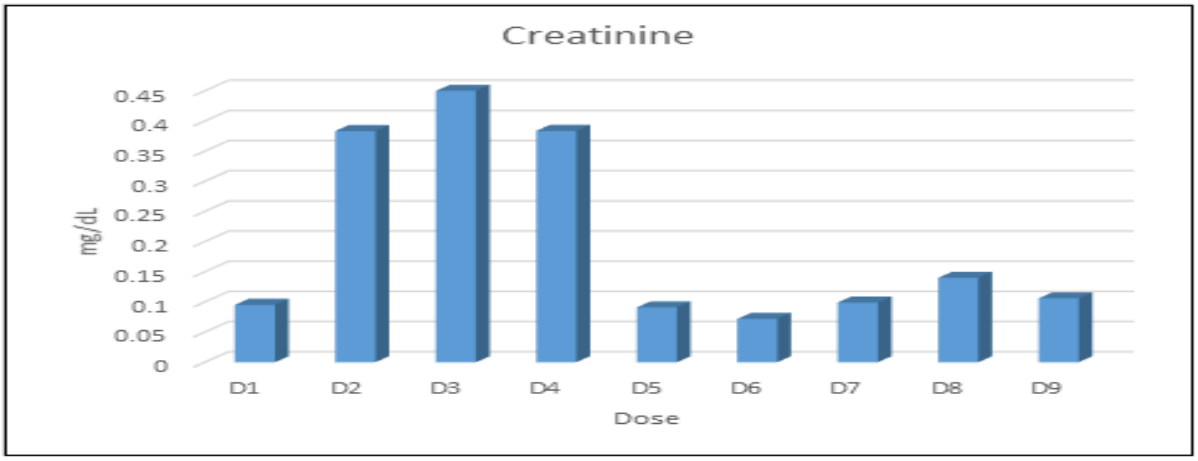

Fig. 4. Graphical analysis of creatinine in mice.

That's why it emphasizes the used to increase energy, strength, and acts as an adaptogen that functions as a powerful immunostimulator. These are prove to retain the health & vitality of people and also heal illness without causing poisoning. (Bhattacharya and uruganandam, 2003). Thus, the above results show that the use of these traditional plants has a logical and scientific foundation.

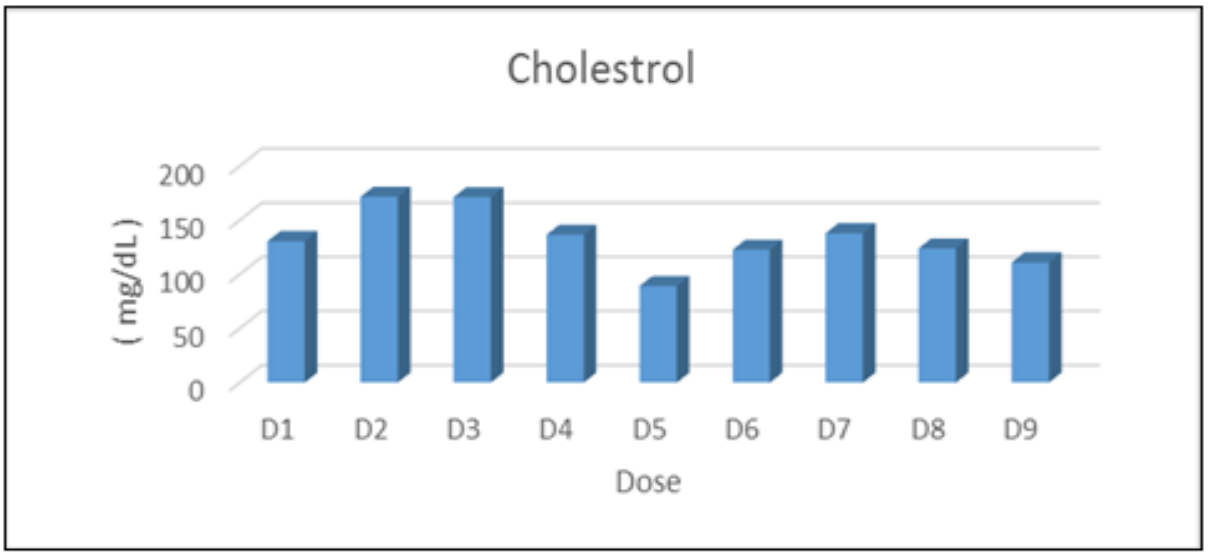

Fig. 5. Graphical analysis of cholesterol in mice.

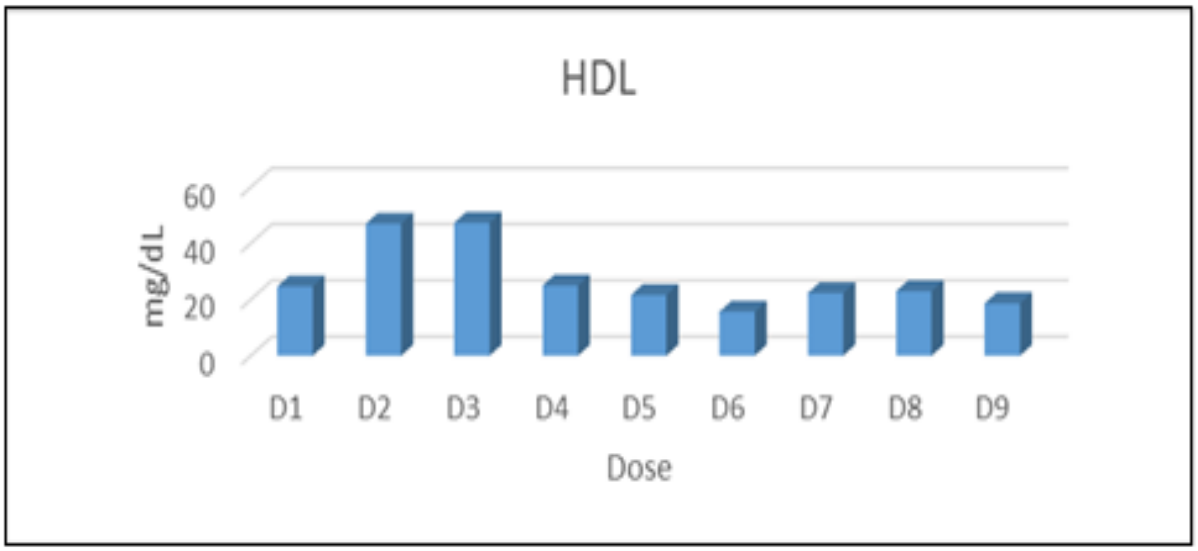

Fig. 6. Graphical analysis of HDL in mice.

Qualitative as well as quanitative analysis of phytochemicals of *Withaniasomnifera and Cnidiummonnieri* cofirm their presenc and and the intake of these phytochemical by diabetic mice support the results and antidibatic and anti-oxidant activity of these plants. Streptozotocin is often used to cause diabetes mellitus in experimental animals. It is usually recognized that the cytotoxicity generated by streptozotocin relies on DNA alkylation and subsequent activation of poly (ADP-ribose) synthetase, which produces fast and deadly depletion of NAD in pancreatic islands. Several lines of proof show that free radicals may play a key part in the cause of β-cell harm and the diabetic impact of streptozotocin. W. somnifera and C. monnieri treatment to diabetic mice reduced the elevated blood glucose level thereby showing its antihyperglycemic activity (Bharti *et al.,* 2012). High blood glucose levels

cause microvascular (neuropathy, retinopathy, nephropathy) and macrovascular complications (stroke, coronary artery disease). There are several phytochemicals like flavonoids, alkaloids, steroids, glycosides and many other bioactive compounds that are present in leaves and roots are extracted and purified from these plants and use for the treatment of diabetes and other disorder that occurred due to diabetes.

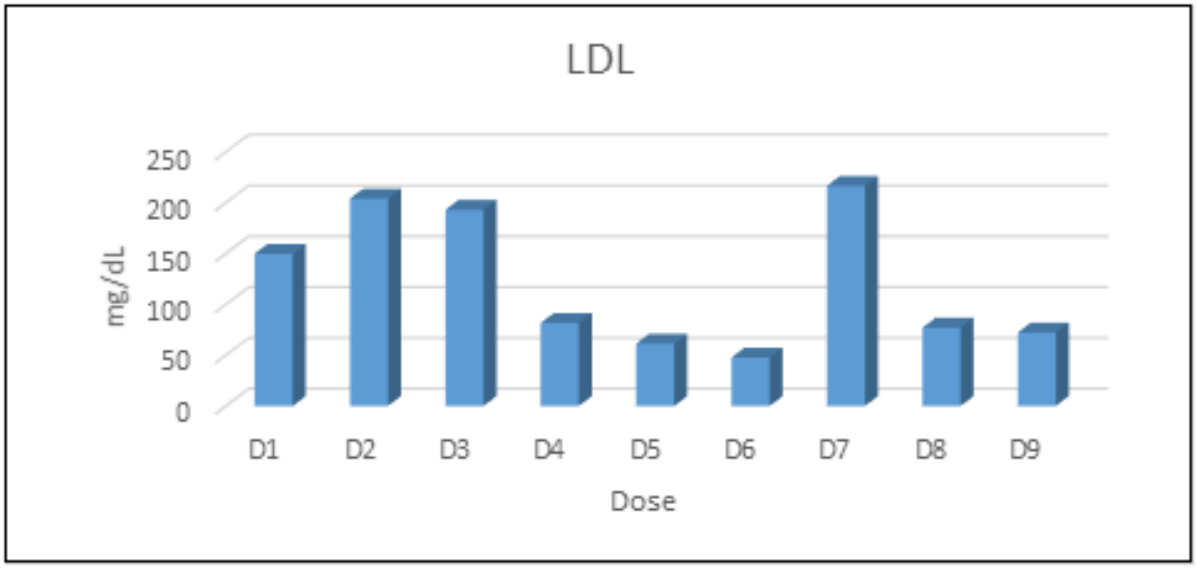

Fig. 7. Graphical representation of LDL in mice.

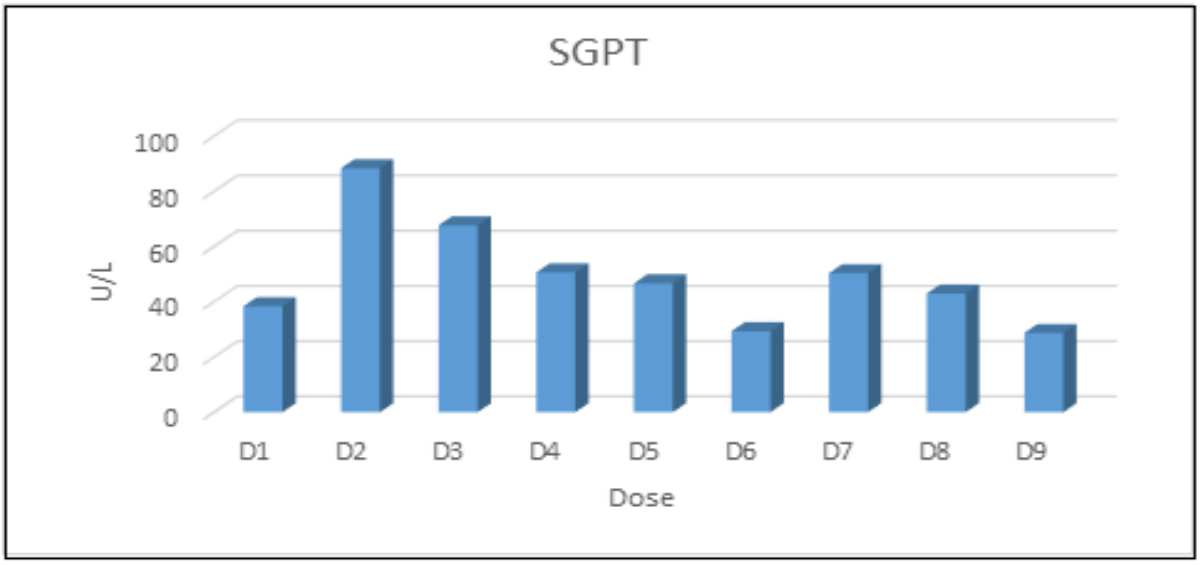

Fig. 8. Graphical representation of SGPT in mice.

In the case of noninsulin-dependent diabetes mellitus (NIDDM), despite elevated insulin levels (hyperinsulinemia), glucose levels in NIDDM control rats were higher than in NIDDM-treated rats. However, WS therapy is efficient in lowering NIDDM rat insulin levels, thereby stopping hyperinsulinemia. It appears that WS has an antihyperglycemic impact by stopping hyperinsulinemia (Anwer *et al.,* 2008).

Overall these plants have the potential to cure all types of diabetes and this research proved it very well. Moreover, if these findings are extrapolated in humans, *Withania somnifera and Cnidium monnieri* may demonstrate helpful in the therapy and/or avoidance of hyperinsulinemia, impaired glucose tolerance and insulin resistance, as well as being an efficient means of regulating glucose levels.

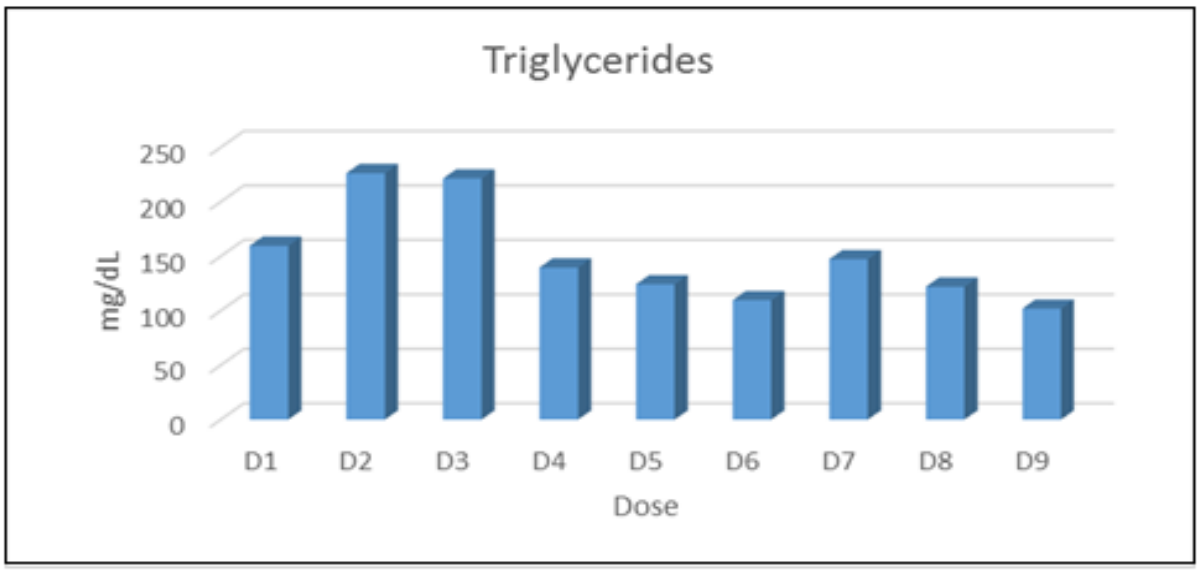

Fig. 9. Graphical analysis of triglycerides in mice.

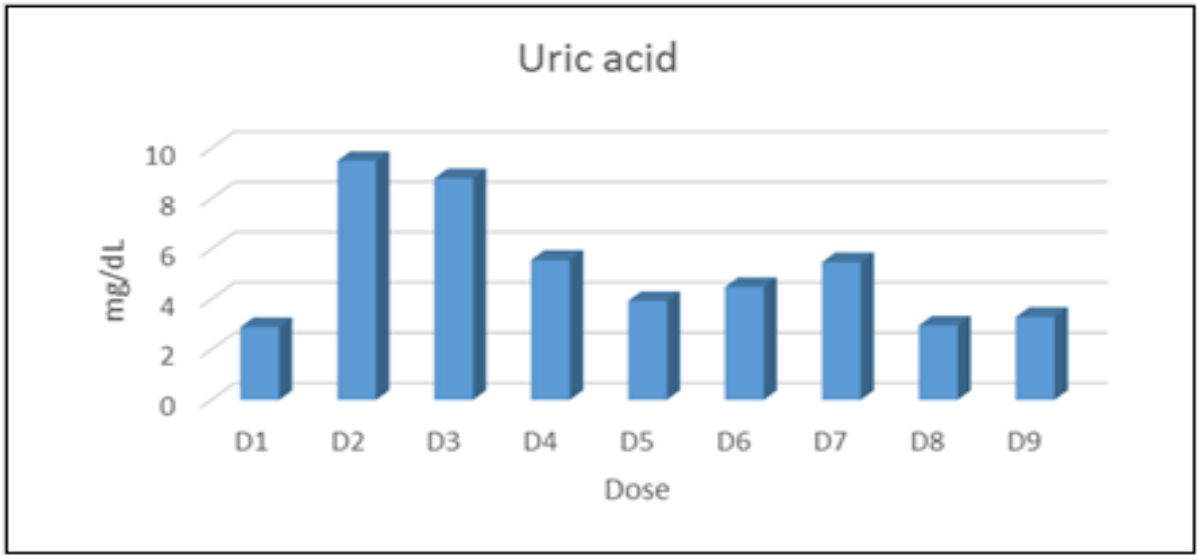

Fig. 10. Graphical analysis of uric acid in mice.

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