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Antidiabetic activity of *Cicer arietinum* in alloxan induced mice

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ABSTRACT

A study of ancient literature indicates that diabetes is fairly well known and well conceived as an entity in India. Health is a birth right of every individual and there is increasing evidence that the dietary habits of people are important determinants of health. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations. The chickpea (*Cicer arietinum*) is an edible legume of the family Fabaceae, subfamily Faboideae. They are high in protein and one of the earliest cultivated vegetables. These seeds were freshly isolated and its methanol extracts were used to check its antidiabetic activity on alloxan induced diabetic mice. Methanolic extracts of *Cicer arietinum* seeds exhibited significant antihyperglycemic activities in alloxan-induced diabetic mice. These extracts showed improvement in parameters like body weight and lipid profile as well as regeneration of beta cells of pancreas and so might be of considerable value in diabetes treatment.

KEYWORDS

Alloxan;
Methanolic extracts;
Antihyperglycemic.

INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to coma and, in absence of effective treatment, death. Bioactive compounds are constituents that are found in certain foods[1]. A wealth of scientific literature from numerous types of epidemiological and case controlled studies have identified the potential relationships between bioactive compounds (or "functional" components) and their protective effects against hypertension, cardiovascular disease, cancer, and other health conditions. Bioactive compounds vary widely in chemical structure and function. For example, plant phenolics are a large and diverse group of phenolic compounds present

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in all plants which include cathechins, isoflavones, anthocyanins, phenolic acids, etc[1]. Alloxan is a toxicglucose, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. This causes an insulin -dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generatesoxygen species(ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction[2].

MATERIALS AND METHODS

Fresh seeds of the selected plant *Cicer arietimnum* having medicinal value were collected from Arunachal Pradesh, India. The seeds were dried in a Hot air oven at 52-55^{o}C for three days. After drying, they were ground well using mechanical blender into fine powder and then transferred into airtight containers for future studies. The fine powder was then extracted overnight by stirring with 10 volumes of 75 % methanol. Supernatant was collected after centrifuging at 3,000 rpm for 10 minutes and named as supernatant I. The pellet is again extracted overnight by stirring with 10 volumes of 75 % methanol. Supernatant was collected at 3,000 rpm for 10 minutes and named as supernatant II. Both the supernatant are mixed and the solvent was evaporated to dryness at 45^{o}C in hot water bath. The yield of the extract was 10% which was used for subsequent assays.

Administration of chickpea extract

For animal administration the Chickpea extract was orally administrated.

Induction of diabetes

Freshly prepared solutions of alloxan monohydrate (Sigma Aldrich Chemicals, Pvt., Ltd., India.) were dissolved in sterile saline at doses of 150 mg/kg body and injected intraperitoneally into the overnight fasted mice. After 72 hours of alloxan injection, the mice with the blood glucose range of above 250mg/dl were considered as the ones with successful induction of diabetes and used for experimental study[3].

Preparation and administration of the standard drug

A standard drug used for the treatment of diabetes namely Metformin was used for treatment. Freshly prepared solutions were obtained by dissolving Metformin in phosphate buffer saline at doses of 7.5mg/dose/animal and then administered orally.

Animals

A special variety of mice mainly inbred for experimental purposes commonly known the BALB/C mice around 6-8 weeks, weighing 23-28g, were purchased from Pasteur Institute, breeding section, Coonoor. The animals were housed in ventilated plastic cages at 37±1°c, 40±10% humidity, and 12-12-h light-dark cycles during the experimental period. The animals were fed with normal mouse chow (Sai Feeds, Mumbai, India), and given water *ad libitum.*

Experimental design

The animals were divided into four groups of six animals each and discussed as follows:

Group 1: Normal Control animals, without any treatment (Healthy control)

Group 2: Animals induced with Alloxan with a dosage of 150mg/ kg body weight (Diabetic control).

Group 3: Animals induced with Alloxan received Chickpea extract for a consecutive period of 10 days (Induced + Chickpea Extract).

Group 4: Animals induced with Alloxan received standard drug named Metformin (7.5 mg/ mice) dissolved in Phosphate buffer saline and administered orally for a consecutive period of 10 days (Induced +Standard drug).

After the experimental study, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected by Heart puncture and serum was separated by centrifugation (for 10 minutes at 3000 rpm). The se-

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TABLE 1 : Effect of Chickpea extract on body weight in alloxan induced mice

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Control	25.53 ± 2.08	26.11 ± 2.23	26.76 ± 1.93	27.28 ± 1.76	27.83 ± 1.96	28.22 ± 1.96
Diabetic Control	21.55 ± 1.28	21.33 ± 1.31	21.59 ± 2.34	20.78 ± 1.69	20.61 ± 1.88	20.47 ± 2.00
Diabetic Induced+Extract	22.67 ± 1.85**	24.18 ± 3.42^{ns}	24.83 ± 2.24^{ns}	25.80 ± 2.63**	26.25 ± 2.55**	26.30 ± 2.03*
Diabetic Induced+Standard	19.14 ± 3.38	18.51 ± 2.59	18.23 ± 2.26	18.33 ± 2.32	18.41 ± 2.29	18.46 ± 2.39

rum was then collected and used for biochemical studies.

Antidiabetic activity of chickpea

Estimation of body weight

The Body weight of the mice was weighed for 0,2,4,6,8 and 10th day

Estimation of blood profile

Effect of blood glucose

The blood glucose level is determined using Trinder`s method with the help of a kit. The blood collected is centrifuged and the serum is used for the experiments.

Estimation of lipid profile

Effect of total cholesterol, effect of triglycerides

The serum was collected from mice in fasting state without any anticoagulant and the lipid profile test was carried out by Pierre et al. 1997 method using Cholesterol kit.

Effect of total serum protein

The serum was collected from mice in fasting state without any anticoagulant. Total protein estimation kit was obtained from Span Diagnostics and total serum protein was estimated.

Estimation of hepatitic profile

Effect of alkaline phosphatase

King`s method is used to determine the Alkaline Phosphatase. Serum collected is to be unhaemolysed and the working buffer solution is prepared with 2.2ml of purified water in buffered substrate for estimation.

Effect of aspartate aminotransferase (AST)

For in vitro determination of Aspartate aminotransferase (AST) in serum/ plasma the Reitman and Frankel Method was used.

Estimation of renal profile

Effect of uric acid

For in vitro determination of uric acid in serum/ plasma the Uricase Methodology is used. The unhaemolysed serum is collected and the experiment was carried out.

Effect of creatinine

Jaffe`s method is used for in vitro quantitative determination of Creatinine in serum. The unhaemolysed serum was collected and the experiment is carried out.

RESULTS AND DISCUSSION

Estimation of body weight

The antidiabetic effects of *Cicer arietinum* extract on the fasting Alloxan induced mice is as shown in TABLE 1 and Figure 1. Body weight was found to be significantly increasing when compared to the drug Metformin with a S.D of 22.67 ± 1.85 to 26.30 ± 2.03 and P value of (P<0.05-0.01) from the 0th day to 10th day of post induction.

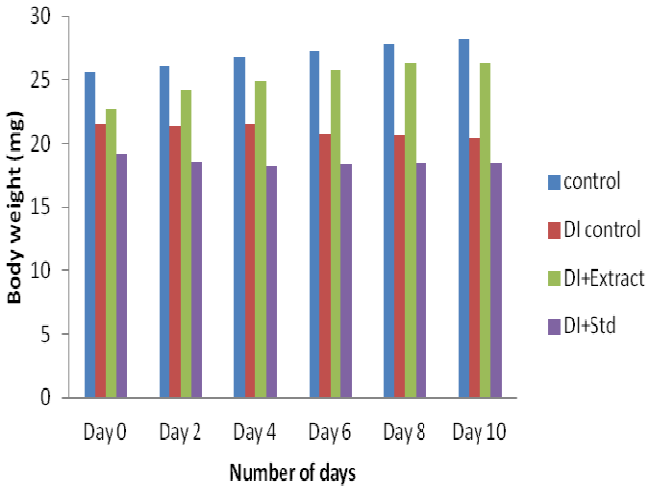

Figure 1 : Effect of chickpea extract on body weight in alloxan induced mice

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TABLE 2 : Effect of chickpea extract on serum glucose level in Alloxan induced mice

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Control	80.81 ± 6.42	83.03 ± 5.36	84.69 ± 6.81	86.36 ± 7.47	867.35 ± 6.86	86.37 ± 7.86
Diabetic Control	305.5 ± 13.67	276 ± 20.36	226.5 ± 20.75	216.66 ± 22.73	212.5 ± 12.81	217.16 ± 17.79
Diabetic Induced+Extract	270.58±14.39^{ns}	226.96 ±16.84^{ns}	213.41 ± 7.13*	173.83 ±6.59**	110 ± 5.17**	94.96 ± 2.40*
Diabetic Induced+Standard	257.66 ± 23.35	222.89 ± 7.93	163.66 ± 3.37	142.87 ± 7.26	134.70 ± 6.41	114.5 ± 9.35

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01and Non Significant when compared to Standard n=6**

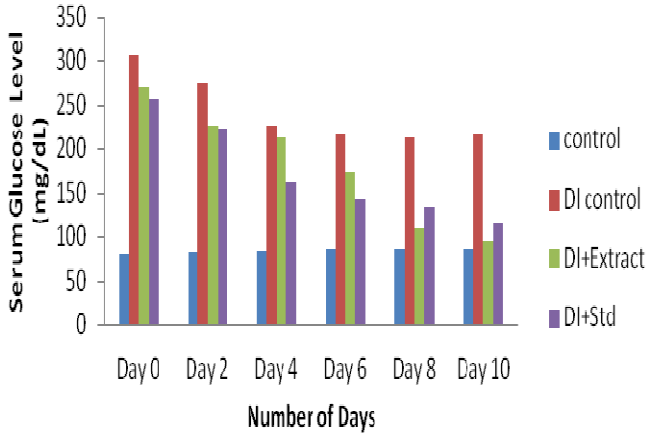


Figure 2 : Effect of chickpea extract on serum glucose concentration in Alloxan induced mice

Estimation of blood profile

Effect of chickpea extract on serum glucose level

The effect of chickpea extract on serum glucose level in Alloxan induced mice is shown in TABLE 2. Glucose level was found to be significantly increasing after Alloxan administration. Administration of extract produced a significant decrease in the serum glucose value when compared to the drug Metformin with arrange of 270.58 ± 14.39 to 94.96 ± 2.40 with a P value of (P<0.05- 0.01) There was a greater significance level observed between the extract treated mice and the Metformin treated.

Effect of total proteins

The result of the effect of chickpea extract on total protiens on Alloxan induced mice is shown in TABLE 3 and Figure is shown in Figure 3. The extract treated animals showed a greater significant value of P <0.01and a S.D of 11.06 ± 1.94**. The extract treated animal showed better significance than diabetic control and Metformin drug.

Estimation of lipid profile

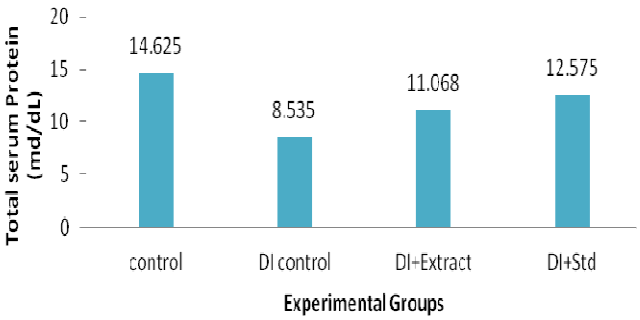
Effect of triglycerides

TABLE 3 : Effect of chickpea extract on total protiens in Alloxan induced mice

Control	Diabetic Control	Diabetic Induced +Extract	Diabetic Induced +Standard
14.62±4.08	8.53 ± 0.31	11.06±1.94**	12.57 ± 1.76

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01and Non Significant when compared to Standard n=6**

Figure 3 : Effect of chickpea extract on total serum protein of Alloxan induced mice



The effect of Chickpea on Triglycerides in Alloxan treated mice is shown in TABLE 4 and in the Figure 4. There is an extreme significant level observed between the extract treated and diabetes induced mice. The S.D value is 122.20 ± 18.16^{ns}. However, the result did not show a significant value with the drug Metformin.

Effect of total cholesterol

The effect of chickpea extract on total proteins in Alloxan induced mice is shown in TABLE 5 and Figure 5. The extract treated animals showed a non significant value of (P<0.05- 0.01) and a S.D value of 124.66 ± 9.21^{ns} when compared with the healthy control and Metformin drug.

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01**and Non Significant when compared to Standard n=6.

Estimation of hepatic profile

TABLE 4 : Effect of Chickpea extract on Triglycerides in Alloxan induced mice

Control	Diabetic Control	Diabetic Induced +Extract	Diabetic Induced +Standard
86.61 ± 12.94	225.22±29.46	122.20±18.16 ns	123.37 ±19.47

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01and Non Significant when compared to Standard n=6**

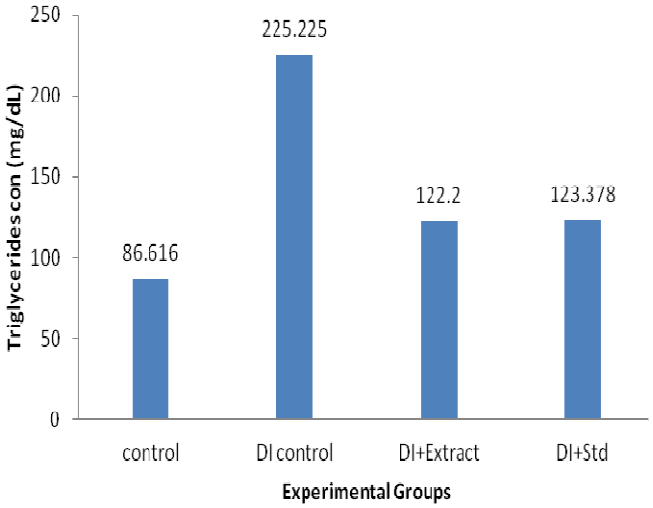


Figure 4 : Effect of chickpea extract on triglycerides in Alloxan induced mice

TABLE 5 : Effect of Chickpea extract on cholesterol in Alloxan induced mice

Control	Diabetic Control	Diabetic Induced +Extract	Diabetic Induced +Standard
85.72± 8.05	234.43±25.73	124.66±9.21 ns	116.49 ± 16.45

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01and Non Significant when compared to Standard n=6**

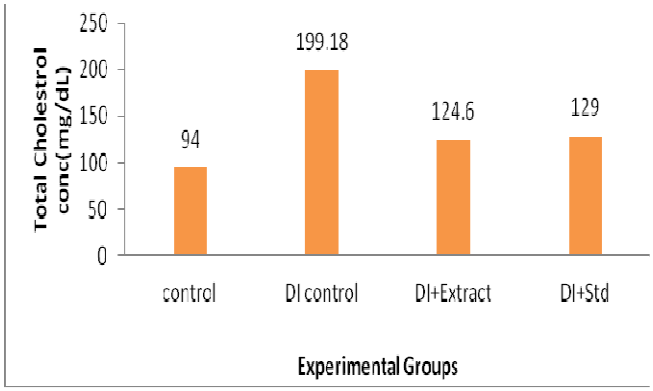


Figure 5 : Effect of Chickpea extract on Total Cholesterol of Alloxan induced mice

TABLE 6 : Effect of chickpea extract on alkaline phosphatase in Alloxan induced mice

Control	Diabetic Control	Diabetic Induced +Extract	Diabetic Induced +Standard
8.47 ± 0.90	11.93 ± 2.06	9.20 ± 0.67**	12.81 ± 2.02

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01and Non Significant when compared to Standard n=6**

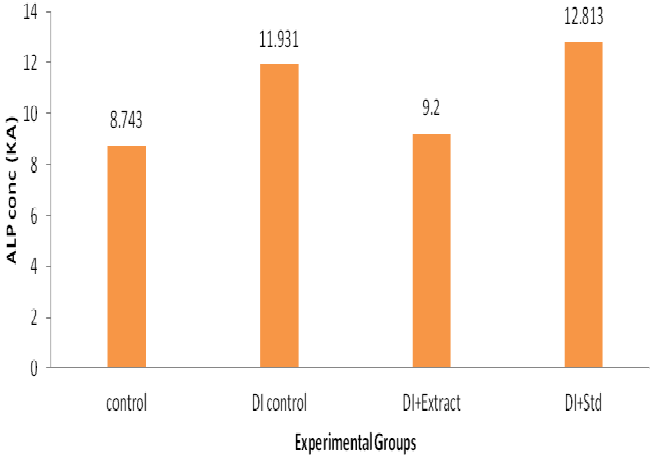


Figure 6 : Effect of chickpea extract on ALP in alloxan induced mice

TABLE 7 : Effect of chickpea extract on GOT in Alloxan induced mice

Control	Diabetic Control	Diabetic Induced +Extract	Diabetic Induced +Standard
84.35 ± 3.08	98.86 ± 9.02	87.81±13.13 ns	122.36 ±12.81

Data are expressed as mean ±S.D. Significant at P<0.05*,P<0.01and Non Significant when compared to Standard n=6**

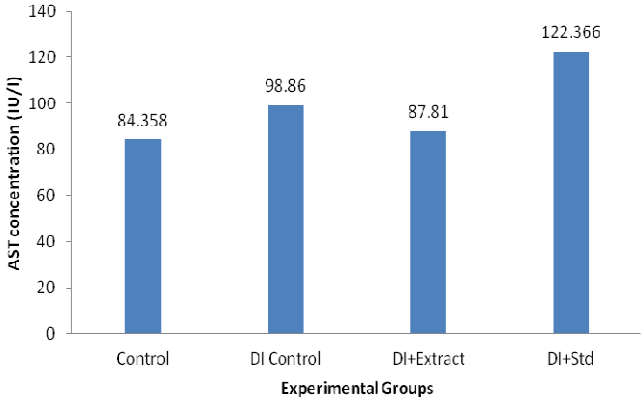


Figure 7 : Effect of chickpea extract on AST (GOT) in Alloxan induced mice

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Effect of alkaline phosphatase

The result of the effect of chickpea extract on Alloxan induced mice is shown in TABLE 6 and Figure 6. The extract treated animals showed a significant value of (P<0.05- 0.01) and S.D value of 9.20 ± 0.67**when compared with the healthy control and Metformin drug.

Effect of aspartate aminotransferase (AST)

The effect of chickpea on Aspartate aminotransferase (AST) level in Alloxan induced mice is shown in TABLE 7 and Figure 7. There is an extreme significant level observed between the extract treated and diabetic control mice with P<0.01 and S.D value of 87.81 ± 13.13. However, the result did not show a significant value with the drug Metformin.

Effect of alanine aminotransferase (ALT)

The effect of chickpea on Alanine aminotransferase (ALT) level in Alloxan induced mice is shown in TABLE 8 and in Figure 8. There is an extreme significant level observed between the extract treated and diabetic control mice with P<0.01and a S.D value of 34.76 ± 2.89 ^{ns}. However, the result did not show a significant value with the drug Metformin.

TABLE 8 : Effect of chickpea extract on GPT in alloxan induced mice

Control	Diabetic Control	Diabetic Induced +Extract	Diabetic Induced +Standard
26.34 ±1.94	59.62 ±5.80	34.76±2.89 ^{ns}	35.60±3.24

Data are expressed as mean ±S.D. Significant at P<0.05*,P<0.01and Non Significant when compared to Standard n=6**

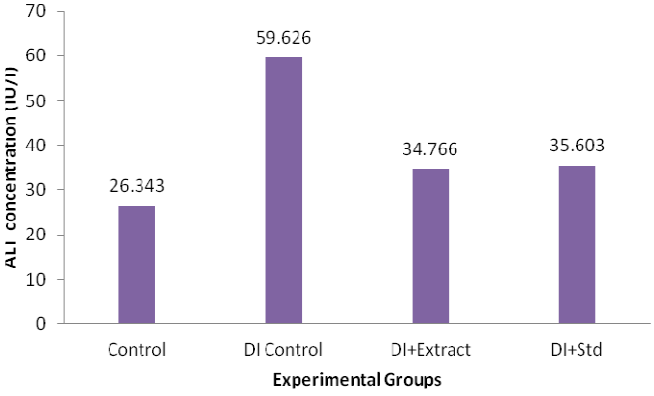

Figure 8 : Effect of chickpea extract on ALT (GPT) in Alloxan induced mice

TABLE 9 : Effect of Chickpea extract on Creatinine in Alloxan induced mice

Control	Diabetic Control	Diabetic Induced +Extract	Diabetic Induced +Standard
1.41 ± 0.51	2.08 ± 0.05	1.46 ± 0.46^{ns}	1.28 ± 0.26

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01and Non Significant when compared to Standard n=6**

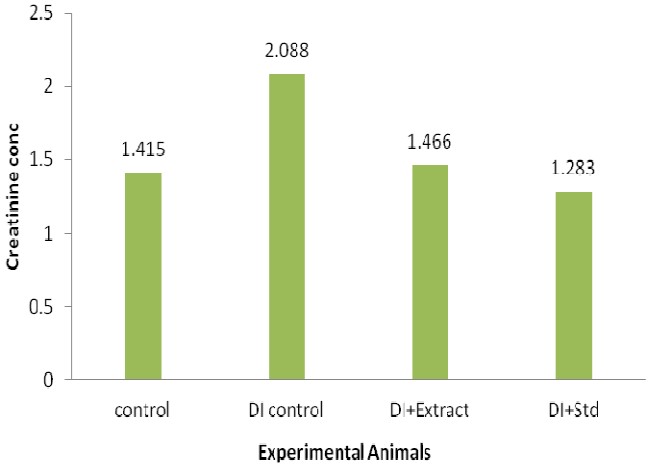

Figure 9 : Effect of chickpea extract on Creatinine in Alloxan induced mice

Estimation of renal profile

Effect of creatinine

The effect of chickpea on Creatinine level in Alloxan induced mice is shown in TABLE 9 and in Figure 9. There is an extreme significant level observed between the extract treated and diabetes induced mice with P<0.001and S.D value of 1.46 ± 0.46^{ns}. However, the result did not show a significant value with the drug Metformin.

Effect of uric acid

The effect of chickpea extract on Uric Acid in Alloxan induced mice is shown in TABLE 10 and in Figure 10. The extract treated animals showed a significant value of (P<0.05- 0.01) when compared with the healthy control and Metformin drug. The P value is < 0.01 and the S.D value is 6.93 ± 0.25** and the value is considered extremely significant.

Alloxan acts as a cytotoxin for beta-cells of the islet of langerhans, causes diabetes by inducing cell necrosis[9]. The Reactive Oxygen Species

TABLE 10 : Effect of chickpea extract on uric acid in Alloxan induced mice

Control	Diabetic Control	Diabetic Induced +Extract	Diabetic Induced +Standard
10.36 ± 0.62	17.33 ± 2.48	6.93 ± 0.25**	11.23 ± 1.67

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01and Non Significant when compared to Standard n=6**

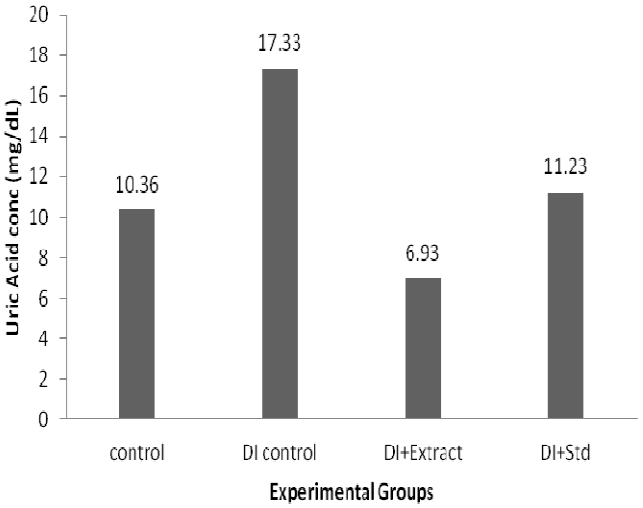

Figure 10 : Effect of chickpea extract on uric acid in Alloxan induced mice

mediates the cytotoxic action with the increase in cytosolic calcium concentration, leading to rapid beta-cells destruction[8]. These results into decreased insulin secretion and elevated blood glucose level[7]. This experimental study reveals that Alloxan- treated mice received soybean extracts showed lower blood glucose level as compared to the diabetic control group which may be due to the possibility that few of beta cells are still surviving and stimulated by extract components which releases insulin.

The extracts exhibited significant reduction of serum cholesterol level in alloxan-treated mice. The abnormal high concentration of serum lipids in the diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots[10].

Diabetes causes disturbance in renal function so the blood creatinine level is elevated[7]. Extract treated group exhibited reduction in serum Creatinine level that indicates that extract may exert its effect on renal function.

Uric acid is one of the main antioxidant present in the body and diabetes causes reduced levels of uric acid [13].

The extract reflects the antioxidant potential as it reduced the oxidative stress and increase in uric acid level.

Proteolysis, lipolysis and acute fluid loss during diabetes pave the way for weight loss[12]. The weight gain in extract treated groups reflects the correction of body metabolism. Results reveal that the chickpea extract exhibits the antidiabetic activity in a dependent manner.

Albumin is tested to check how well the liver andkidneysare working and if the dietenough protein. Globulin is tested to determine the chances of developing an infection. Results show that there is a significant increase in the protein level which means that the liver and kidney functions are normal.

Presence of Triglycerides may be probably due to in obesity and fat deposits which may be due to diabetes. The extract showed a significant result which shows that there is no fat deposition.

The alanine aminotransferase (ALT) blood test is typically used to detect liver injury. It is often ordered in conjunction withaminotransferase (AST)as part of apanelscreen for and/or help to diagnose thedisease. AST and ALT are considered to be two of the most important tests to detect liver injury, although ALT is more specific than AST. Sometimes AST is compared directly to ALT and an AST/ALT ratio is calculated. This ratio may be used to distinguish between different causes of liver damage. The level of AST and ALT are analyzed and found to be normal.

CONCLUSION

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations.

The chickpea (*Cicer arietinum*) is an edible legume which belongs to the family Fabaceae,

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subfamily. Chickpeas are high in one of the earliest cultivated vegetables. These seeds were freshly isolated and its methanol extracts were used to check its antidiabetic activity on alloxan induced diabetic mice.

Body weight and blood glucose levels were checked on even days, where as the lipid, renal and hepatic levels were estimated on the last day by cardiac puncture. A steady increase of body weight was noticed throughout the period. The group treated with chickpea extracts showed that their blood glucose levels significantly lowered in comparison to the standard drug that was administered. Stable levels of total protein, total cholesterol, triglycerides, uric acid, creatinine, SGPT and SGOT ($P < 0.05 - 0.01$) showed that although diabetes affects the liver and kidney. Estimated values suggested that these organs remained stable and completely functional throughout the period of the study.

Methanolic extracts of *Cicer arietinum* seeds exhibited significant antihyperglycemic activities in alloxan-induced diabetic mice. These extracts showed improvement in parameters like body weight and lipid profile as well as regeneration of beta cells of pancreas and so might be of considerable value in diabetes treatment.

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