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HYPOGLYCEMIC ACTIVITY OF *CANNABIS SATIVA* L. ON ALOXAN INDUCED DIABETES

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ABSTRACT

Diabetes mellitus is commonly referred to as "diabetes." It's a metabolic condition caused by a mix of inherited and environmental factors that leads to unusually high blood sugar levels. The body's blood glucose level is regulated by a complicated interaction of various substances and hormones. For this purpose rabbits were used as experimental animals, as all mammals have same characteristics so, rabbits were used. The results of research proved significant reduction in sugar level on 15 days of trials. Results depicted that T_2-group treated with 1g of leaf extract from *Cannabis* showed maximum decrease in blood glucose level i.e; 170±1.15 mg/dl. The body weight was also decreased in T_2-group i.e; 1.15±0.01 kg, as compared to other groups. . Serum insulin levels improved in both treatment groups, although they improved the most in the T2-group on the 15th day of treatment, reaching 5.940.01 pmol/L. The researchers discovered that an ethanolic extract of *Cannabis sativa* L. had a significant anti-hyperglycemic potential. (less than allopathic medicines) which can be used to cure in diabetes mellitus, thus justifying that the novel allopathic and herbal drug can be produced and optimized from this plant, from AJK territory. The present investigation showed that the *Cannabis sativa* L. leaves extract can be used efficiently in diabetes.

INTRODUCTION

Diabetes is metabolic diseases that cause high blood sugar level. Diabetes also called "hypoglycemia". The food that we eat is mostly metabolized into sugar or glucose. And this sugar is released in our blood stream. But when this sugar moved up, it activates our pancreas to liberate insulin. Insulin causes the blood sugar to moves into the body cells for energy usage (Alam *et al*. 2005). But in diabetes our body does not made adequate insulin, so when there is no sufficient insulin in our body then more sugar will stay remain in our blood stream. And that's how the diabetes or hypoglycemia is occurred (A. Eidi *et al.,* 2006). There is no cure for this disease yet, but a healthy diet and active living can help in this disease. It is a long lasting disease. It can cause damage to heart and kidney (Levendal and Frost, 2006).

Types of Diabetes

1. Type 1 diabetes: This type is an immunological disease. Our immune system hostilities and demolish our pancreas (the organ that made insulin). The source or cause of this attack is unknown. About 10% people have this type of diabetes.

2. Type 2 diabetes: It happens when our body immune (resistant) to insulin and sugar strengthen into our blood.

3. Pre-diabetes: It happens when blood sugar level of body is more than normal, but this is not more adequate for identification of diabetes type 2.

4. Gestational diabetes: This is high blood sugar in the time of pregnancy. Insulin stop hormones released by amnion (placenta) are a source of this type of diabetes. The common symptoms of diabetes are: More appetite, Increased thirst, losing weight, Periodic or repeated urination, Faint or fuzzy sight of eye, Acute or severe tiredness, Swelling, inflammation, wound or scrape that do not cure or heal (Watson, 2020).

MATERIALS

Alloxan monohydrate (chemical), saline water, distilled water, Blood Glucose determination Kit, Rabbits, Syringes for injection.

METHODOLOGY

Experimental Animal

Male local rabbits (*Oryctolagus cuniculus*) weighing 1.0-1.5 kg were used for this investigation. These were kept in wooden house and were given balanced diet comprising of green leaves, grass and water. A deliberate measure of new sustenance was recharged every day at 9:30 am, 12:30 pm and 5:00 pm.

Collection of Plant Material

The leaves of *Cannabis* were collected from district Bhimber and identified with the help of Mrs. Waheeda Mushtaq, a renowned botanist. Before plucking up the leaves it was checked carefully that leaves should be fresh and green. The material of plant was dried in the shade till the leaves were dried well for grinding. Later the plant material well grinded by using grinder or blender into a powder form and placed in sealed jars with appropriate tagging for later use. The dried specimen was submitted in herbarium of MUST with herbarium no. 2276.

Preparation of Extract

The 250-gram powder of leaves of *Cannabis* was dipped into 700ml of ethanol for seven days. After 7 days the material that was dipped was filtered by Whatmann filter paper to obtain appropriate filtrate. Filtration method was repeated three times to totally finish the plant material. This ethanolic extract was dried up by using rotator evaporator at a temperature lower than 40 ^{0}C. The final residue collected was a thick paste and saved at 4 degrees centigrade. (Ahmed *et al.,* 2019).

This activity was done in the laboratory of MUST (Mirpur University of Science and Technology), Botany Department Bhimber (AJK). These experiments were performed according to the directions of Alam *et al*. (2005)

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Induction of Diabetes in Rabbits

Optimization of dose

To make the rabbits diabetic, various doses of Alloxan were given at first. Animals were separated into five groups for this purpose. The first group received a dose of 60 mg, the second received a dose of 75 mg, the third received an 85 mg dose, the fourth received a dose of 95 mg, and the fifth received a dose of 100 mg. The rabbits in the first group did not develop diabetes. Only one rabbit from the third group, which received an 85mg dosage, developed diabetes and survived. This dose has also been shown to be effective (Alam *et al.*, 2005). Seventy percent of the rabbits in the fourth group, which was given a 100mg dose of Alloxan, died.

Procedure for Injecting Alloxan Monohydrate

The procedure for injecting alloxan monohydrate into the rabbits was followed, as described by Akhtar *et al*. (1981). Xyelene was placed to their ear before to the injection, making their vein more visible and easier to inject. With the help of a 3cc syringe, the required amount of Alloxan was mixed in the measured volume of saline solution and injected into the rabbit's marginal ear vein without wasting a single minute.

To avoid a hypoglycemic attack, 2g of glucose were dissolved in 10cc of distilled water and administered orally to each rabbit prior to Alloxan induction. The dose of Alloxan was carefully calculated based on the weight of the rabbits as well as their overall health. Required dose of Alloxan was added in saline solution to make 5% of Alloxan solution (Ahmed *et al.* 2010)

Calculations

Alloxan can be measured by following method, According to the weight in 85mg/kg ratio:

Weight of rabbits in grams		Weight of Alloxan in mg
1000g requires	=	85mg
1g requires	=	85/1000

1.346 requires = 85/1000*1.346

That is

0.1144g

In which ratio saline solutions added to Alloxan

5% of Alloxan means = 5g Alloxan in 100ml normal saline solution

Weight of Alloxan in mg. normal saline solution in ml.

5000mg. = 1000ml.

1mg. = 100/5000

If requires weight of Alloxan is 128mg then

128mg = 100/5000*128 = 2.56ml.

Their blood glucose levels were measured using a glucometer on the eighth day after receiving the dose. Rabbits were classified as diabetic when their blood glucose level exceeded 350 mg/dl. Before starting treatment, three or four readings were taken; if these readings were above the required level of diabetes (350 mg/dl), then treatment was started.

Treatment of Diabetes

Experimental Design

Rabbits (the experimental animals) were placed into four groups, with each group consisting of four rabbits.

Group I: This group was regarded as a standard control group (NC). There isn't any Alloxan induction or plant extract induction.

Group II: This group was referred to as the diabetes control group (DC). Alloxan was provided to this group; however, they were not given plant extract.

Group III: This group is referred to as the treatment control group (TC). This group received Alloxan injections, and the rabbits of this group were also treated with Glucophge, an allopathic drug that is common in market.

Group IV: This group is referred to as treatment group 1 or trial 1 (T_1). Alloxan was provided (injected) to the rabbits of this group and an oral dose of 0.5g (500mg) of ethanolic extract of the leaves of *Cannabis sativa* was also given to rabbits per day.

Group V: This group is referred to as treatment group 2 or trial 2 (T_2). Alloxan was provided (by injection) to rabbits and they were also treated with an oral dose of 1g (1000mg) of ethanolic extract of the leaves of *Cannabis sativa* per day. (Same method wasalso used by Manikandan *et al.*, 2013)

Procedure Followed

After making rabbits diabetic they were given 1gram dosages of Cannabis sativa's leaf extract. The trial period lasted 15 days, during this time various analyses were carried out. After three days, their diabetes was examined. Their blood samples were taken on the 7th and 15th days and forwarded to the Armed Forces Institute of Pathology (AFIP) Rawalpindi for a serum insulin test. Every third day, their weight was also measured.

Analysis During Treatment

Measurement of Glucose

During diabetic treatment of rabbits, blood glucose level was measured by glucometer and for this purpose rabbit's ear vein was punctured with a needle to get one drop of blood. The drop of blood was collected and dropped o0n glucometer strip. After receiving blood, a reading that shows the blood glucose level was blinked on its screen.

Serum Insulin Test

During the treatment of rabbits, on the 7^{th} and 15^{th} day approximately 3cc. blood sample was taken from a rabbit's thigh vein. Serum was collected using a centrifuge machine and

transported to the Armed Forces Institute of Pathology (AFIP) in Rawalpindi for a serum insulin test (Frisher *et al.* 2010)

Measurement of weight

Throughout the treatment, after every 3rd day the weight of rabbits was also weighed, with the help of a digital weighing machine.

RESULTS

Extract of *Cannabis*

Ethanol was used to make extract of *Cannabis sativa* L. The extract was sticky and slowly flows and it shows shiny black colour.

Measurement of Blood Glucose Level

To evaluate the effects of an extract of Cannabis sativa L. leaves, the blood glucose levels of normal control and treatment groups were monitored every third day for 15 days using a glucometer. The results demonstrated that blood glucose levels in rabbits treated with 0.5g of leaf extract (T_1) was (182±1.452966 mg/dl), the glucose level of the rabbits treated with 1g of extract (T_2) was (170±1.154701 mg/dl) and the glucose level of rabbits treated with Glucophage (Allopathic medicine) was (165±0.942809 mg/dl) are significantly lower than diabetic group (DC) that was (521±0.57735 mg/dl). As shown in table 4.4. Graphical demonstration is given in Figure 1.

Table 1: Blood glucose level.

	NC	DC	TC	T_1	T_2
D0	120±0.333	521±0.57	501±0.47	500±0.33	501±0.66
D3	122±1.15	522±1.15	449±0.94	500±1.15	498±1.15
D6	121±1.15	524±1.15	391±0.47	415±1.45	390±1.15
D9	120±1.16	528±1.15	319±0.94	330±0.88	320±1.15
D12	120±1.73	532±1.15	339±0.94	245±1.15	240±1.15
D15	122±1.45	534±1.15	165±0.94	182±1.45	170±1.15

NC is normal control group, DC is Diabetic control, TC is Treatment control T_1 is treatment with 0.5g extract (Trial 1) and T_2 is treatment with 1g of extract (Trial 2) and D3 is after three days, D6 after 6 days, D9 after nine days, D12 after twelve days and D15 are after fifteen days measurement.

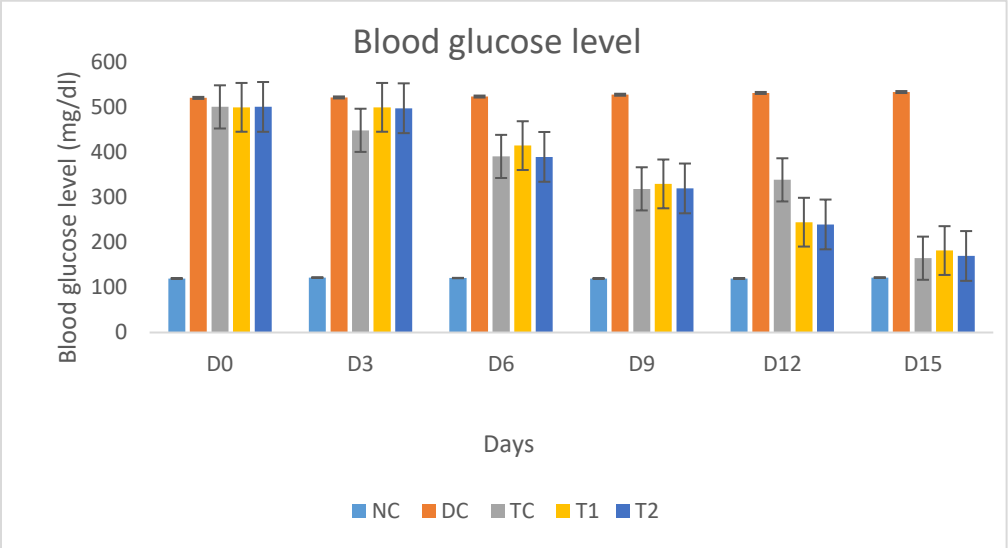

Fig. 1: Graphical representation of blood glucose level

NC is normal control group, DC is Diabetic control, TC is Treatment control T_1 is treatment with 0.5g extract (Trial 1) and T_2 is treatment with 1g of extract (Trial 2) and D3 is after three days, D6 after 6 days, D9 after nine days, D12 after twelve days and D15 are after fifteen days measurement.

Measurement of Weight of Rabbits

For 15 days, the weights of normal control and treatment groups were checked every third day using a digital weighing machine to evaluate the effects of Cannabis sativa L. leaf extract. The result indicated that the weight weighed at day 15^{th} of rabbits treated with 0.5g extract of leaves (T_1) was (1.18±0.01kg), the weight of the rabbits treated with 1g of extract (T_2) was (1.15±0.01kg) and the weight of rabbits, treated with Glucophage (Allopathic medicine) was (1.12±0.03kg). As shown in table 2. Graphical representation is shown in Figure 2.

Table 2: Weight of rabbits

	NC	DC	TC	T_1	T_2
D0	1.33±0.09	1.36±0.01	1.30±0.01	1.33±0.01	1.37±0.01
D3	1.33±0.02	1.42±0.01	1.42±0.02	1.42±0.01	1.43±0.03
D6	1.34±0.02	1.44±0.01	1.30±0.01	1.33±0.01	1.33±0.01
D9	1.34±0.01	1.45±0.01	1.21±0.01	1.27±0.01	1.26±0.01
D12	1.36±0.01	1.47±0.02	1.12±0.01	1.21±0.02	1.18±0.02
D15	1.37±0.02	1.48±0.02	1.12±0.03	1.18±0.01	1.15±0.01

NC is normal control group, DC is Diabetic control, TC is Treatment control, T_1 is treatment with 0.5g extract (Trial 1) and T_2 is treatment with 1g of extract (Trial 2) and D3 is after three days, D6 after 6 days, D9 after nine days, D12 after twelve days and D15 are after fifteen days' measurement.

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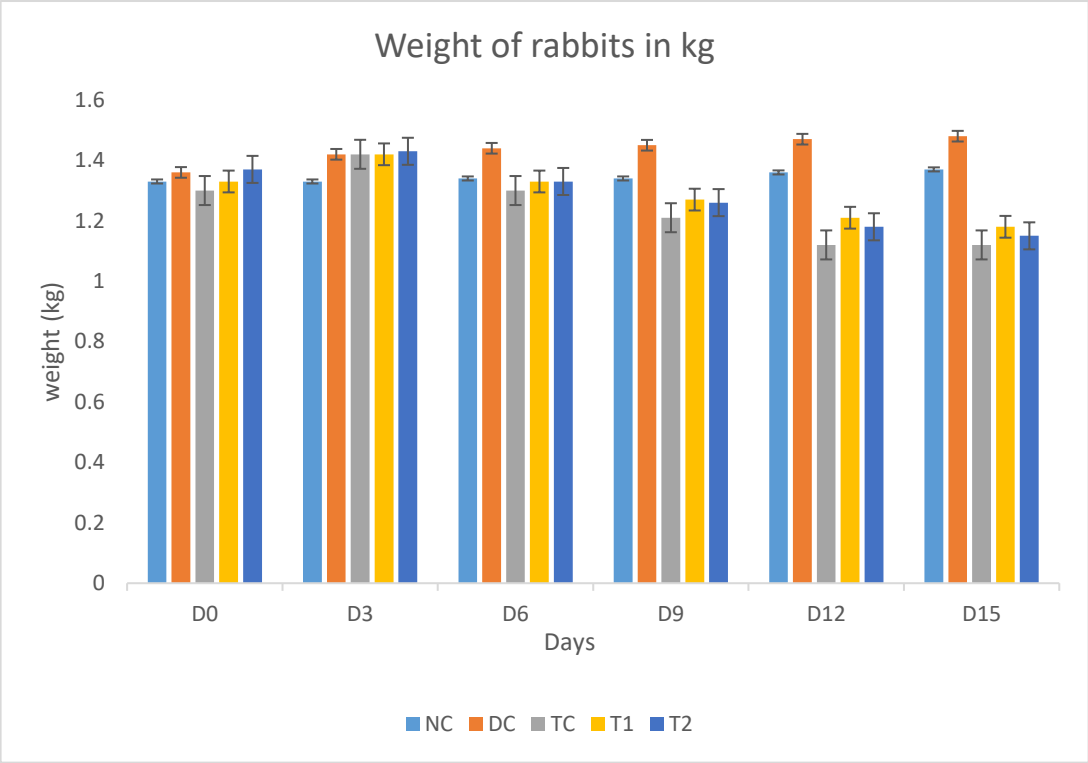

Fig. 2: Graphical representation of weight of rabbits.

NC is normal control group, DC is Diabetic control, TC is Treatment control, T_1 is treatment with 0.5g extract (Trial 1) and T_2 is treatment with 1g of extract (Trial 2) and D3 is after three days, D6 after 6 days, D9 after nine days, D12 after twelve days and D15 are after fifteen days' measurement.

Measurement of Serum Insulin Level

The effect of an ethanolic extract of Cannabis sativa was calculated by measuring serum insulin levels in normal control and treatment groups on the 7th and 15th days of the experiment. The results showed that serum insulin level at day 15^{th} of rabbits treated with 0.5g extract of leaves (T_1) was (5.31±0.01pmol/L) and the serum insulin level of the rabbits that were treated with 1g of leaf extract (T_2) was, (5.94±0.01 pmol/L) and the serum insulin level of rabbits treated with Glucophage (TC) was (6.52±0.01 pmol/L) were significantly higher than diabetic group (DC), that was (0.00±0.033 pmol/L). As shown in table 3.and graphical representation is shown in Fig. 3.

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Table 3: Serum insulin level

	NC	DC	TC	T_1	T_2
D7	7.46±0.01	0.03±0.03	4.43±0.01	3.24±0.01	3.72±0.01
D15	7.61±0.01	0.01±0.01	6.52±0.01	5.31±0.01	5.94±0.01

NC is normal control group, DC is Diabetic control, TC is Treatment control, T_1 is treatment with 0.5g extract (Trial 1) and T_2 is treatment with 1g of extract (Trial 2) and D7 is after seven days and D15 is after fifteen days' measurement. D7 is first checking day and D15 is second checking day.

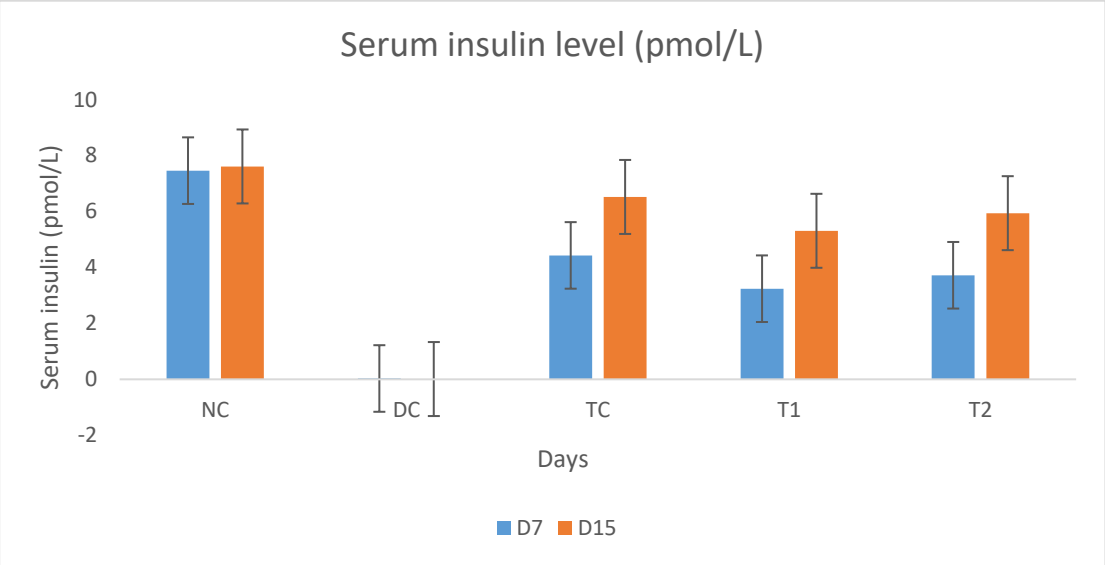

Fig. 3: Graphical Representation of Serum Insulin level of Rabbit.

NC is normal control group, DC is Diabetic control, TC is Treatment control, T_1 is treatment with 0.5g extract (Trial 1) and T_2 is treatment with 1g of extract (Trial 2) and D7 is after seven days and D15 is after fifteen days' measurement. D7 is first checking day and D15 is second checking day.

DISCUSSION

According to new research, alloxan is an effective pro-oxidant that is cytotoxic to ß cells in the pancreatic islets of epidermal DCs (Langerhans) (Shanti, 1994). Alloxan has been demonstrated to inhibit the activity of a calcium and calmodulin-dependent protein kinase, which inhibits insulin production (Katzung 1993).

A slight fluctuation in blood glucose level was seen in the control group of rabbits in our experiment, which could be due to meal variance. A 70% drop was seen in the group of rabbits treated with 1g of glucophage and in the other group that is treated with 1g of leaves extract 65% drop was observed in glucose level during the interval of 15 days (Bilal *et al.* 2018)

Body weight is also affected by *Cannabis sativa* leaf extract. In group 1 which was considered as normal control 4.4% increase in the weight was noticed. In the diabetes control group, there was a 1.35 percent weight loss due to a change in glucose levels. The group treated with glucophage saw an 8 percent decrease in body weight, while the group treated with leaf extract saw a 35 percent decrease in body weight. By lowering their weights, all of the chemicals present5 in Cannabis sativa normalize blood glucose levels. As a result, people can lower their weights and so normalize their blood glucose levels. (Ramachandran *et al*., 2012).

The drop in blood glucose and blood insulin was related to the destruction of -cells, which was also tested. On the 7th and 15th days of the experiment, serum insulin was measured. The blood insulin level of the normal control group did not change significantly, whereas the insulin level of the diabetic control group increased to 0.001 and remained stable after 15 days. Between the 7^{th} and 15^{th} day, the group treated with glucophage showed a 13 percent improvement. There is a 25% improvement in serum insulin levels in the group that is given 1g of leaf extract. Many studies have found that blood glucose levels are linked to cells, and that insulin levels are likewise influenced by these cells. This level fell in Alloxan diabetic rabbits due to cell damage. The gradual improvement of cells was noticed with the usage of a plant-

based ingredient formula that improved blood glucose and serum insulin levels. (Wadood *et al*., 2007).

CONCLUSION

It is concluded that all the compounds that are present in *Cannabis* are effective remedy of diabetes like sterols, alkaloids, tannins and flavonoids. Leaf extract reduces the blood glucose level and body weight also. Its continuous use can repair the β- cells of pancreas. There are many side effects of allopathic medicines so it is highly recommended that one should use the herbs instead of using allopathic medicines.

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