

## ANTI-DIABETIC EFFECT OF CICHORIUM INTYBUS SEEDSEXTRACT IN ALBINO MICE

<sup>1\*</sup>AMNA REHMAN, <sup>2</sup>KAMRAN FAROOQ AND <sup>3</sup>SARA HAYEE

<sup>1</sup>Government M.A.O. College Lahore.

<sup>2</sup>Department of Chemistry, Government M.A.O. College, Lahore.

<sup>3</sup>Department of Zoology, Government Graduate College for Women Samanabad, Lahore.

Corresponding Author email: newton1717@hotmail.com

### خلاصہ

یہ تحقیقی کام البینو چوہوں میں ایم ایس جی (مونوسوڈیم گلوٹامیٹ) کے ذریعے پیدا کی گئی ذیابیطس پر سکوریم اینٹی بس بیجوں کے عرق کا اینٹی ذیابیطس اثر معلوم کرنے کے لیے کیا گیا ہے چوہوں کا یہ گروہ انسانوں سے بہت قریب سے مشابہت رکھتا ہے لہذا اس کا مطالعہ کے لئے انتخاب کیا گیا ہے۔ یہ پایا گیا کہ چکوری بیجوں کے عرق نے ذیابیطس شدہ جانوروں پر بے حد اینٹی ذیابیطس اثر ڈالا ہے اس جڑی بوٹی کے بیجوں کے عرق کے استعمال سے ان کی ذیابیطس کی سطح کو باقاعدگی سے کم کیا گیا۔ نتائج نے ثابت کیا ہے کہ چکوری بیجوں کے عرق کو بطور اینٹی ذیابیطس دوا بغیر کسی مضر اثرات کے استعمال کیا جاسکتا ہے۔

### Abstract

This research work was performed to investigate the anti-diabetic effect of *Cichoriumintybus* seeds extract on MSG (Mono Sodium Glutamate) induced diabetic Albino mice. This group of mice is closely resembled to human beings, therefore it was selected for the study. It was found that chicory seeds extract had an immense anti-diabetic effect on MSG-induced diabetic animals and their diabetic levels were lowered regularly with the use of this herbal seeds extract. The results confirmed the use of *C. intybus* seeds extract as an anti-diabetic herbal medicine without any harmful side effects.

**Keywords:** *Cichoriumintybus*, Mono Sodium Glutamate, Albino.

### Introduction

From the times of Hazrat Adam (A.S.) till date, all human beings are dependent on plants for food, fuel, fragrances, medicines and flavors etc. medicinal plants have always been used for the treatment of various diseases in man. In the past history of man, it was a common practice to grow herbal plants in lawns and gardens. Now days, people are dependent on manmade synthetic drugs (Racadio *et al.*, 2008). Diabetes is one of the deadliest diseases affecting millions of people and leading to associated complexities like micro-circulatory retinopathy, neuropathy, atherosclerosis and many more. It has been observed that 33 million people in Pakistan are affected with type 2 diabetes, the third largest population worldwide (Zulfiqar *et al.*, 2022).

Some reports have revealed that 11 million adults in Pakistan have glucose intolerance while about 8.9 million people have diabetes undiagnosed. Diabetes mellitus is a disease involving hyperglycemia, high metabolic rates and lipoprotein abnormalities. High oxidative stress along with scavenging enzymes cause damage to beta cells of pancreas. Today, diabetes mellitus is considered as third most fatal disease and leading cause of death (Vivek *et al.*, 2010). Number of people getting diabetes mellitus are increasing day by day due to less physical activities, obesity, heredity causes and use of food additives (King, *et al.*, 1993). Diabetes causes increased weight gain or loss, sudden increase or decrease in levels of blood glucose, increased hunger, dehydration and blindness (Ramachandran, *et al.*, 1999). The rate of people getting this fatal disease is increasing day by day. The ratio may reach from 8.3% to 9.9

% affecting 366 to 522 million of people in the world. Its rate is increasing day by day in developing countries like China, India and Pakistan. Chances of males getting diabetes are twice as compared to females. A recent data revealed that prevalence rates were 9.9% (95% CI, 8.8–11.0%) among men, and 11.6% (95% CI, 10.0–13.1%) among women (Hongyan, *et al.*, 2019). It is essential to cure and control diabetes earlier because its long term affects include sexual dysfunction, blindness, foot ulcers, renal failures and cardiovascular diseases (Karimulla, *et al.*, 2011).

From Asia to Africa, *C. intybus* commonly known as chicory has been used as a traditional medicinal plant. It has been reported earlier that aqueous seed extract of this plant show anti-diabetic effect. In a research on Wister rats, it has been observed that leaf powder of this plant decreased the glucose level to normal level. It has been observed that chicory possesses anti-diabetic activity. An analysis on chicory leaves gives its nutritional

importance as its 100 grams contains 92.5% moisture, 1.65 proteins, 0.15 5 fiber and 4.2 % carbohydrates. It has been observed that *C. intybus* rich in minerals having thiamine, riboflavin, vitamins C, niacin, riboflavin, carotene, iron, calcium and phosphorus. The calorific ratio present in chicory ranges 20-25. Some bitter substances present in it are intbin and lactucin which are mainly present in its flowers. Quality control analysis of seed extract of this plant showed the presence of flavonoid and phenolic components 8.3% and 34.8 % w/w respectively. Also, Chicoric acid is also a new anti-diabetic agent containing both insulin secreting and sensitizing properties which is found in chicory seeds (Ahmad, *et al.*, 2009).

Since diabetes mellitus has become a major health issue globally, its prevention and treatment is very crucial for public health. Many efforts have been put in by scientists to use medicinal plants for its better treatment. Here in this research *Cichorium intybus* seeds extract has been used to treat diabetic mice. Diabetes was induced in Albino mice by using monosodium glutamate (MSG) instead of streptozotocin or alloxan which are usually used for the induction of diabetes. Also, MSG is much cheaper as compared to these above mentioned medicines.

Many allopathic medicines are used to control diabetes, but these medicines have side effects which can cause more danger to the patients especially patients having cardiac micro or macro-vascular issues. Side effects which have been observed are coma, hypoglycemia, blindness etc. The use of synthetic drugs causes more complications in pregnant women during delivery and can also be transferred to fetus. Synthetic drugs are very costly. World health organization recommended natural products that are produced from medicinal plants for the treatment of diabetes because medicinal plants have fewer side effects as compared to the synthetic drug. Medicinal plants have a higher potential for hypoglycemic agents and treat diabetes more effectively.

## Material and Method

Seeds of *C.intybus* were collected from Papar mandi Lahore. The local names used for this plant are Kasni and chicory. Seeds were identified by the botanist of Govt. M.A.O. College Lahore.

The seeds of chicory were shade dried for almost 14 -15 days and then ground into fine powder. An aqueous ethanolic extract of 80% concentration was prepared by mixing 860 mL ethanol and 140 mL distilled water to make a 1000 mL alcoholic aqueous extract. Soxhlet apparatus was used for the hot extraction of seeds. For Soxhlet extraction procedure, thimbles were prepared by cutting a filter paper into a length of 40cm and width of 15cm. In this way ten thimbles were prepared. From the open end of the thimble *C. intybus* seeds powder (approx;140-160g) was filled in a thimble. Almost 1500g of powder was filled in all the ten thimbles and ready for the extraction process. The round bottom flask of the Soxhlet apparatus was filled with ethanol. Apparatus was turned on and run for 6-8h and ethanolic seeds extract was collected by continuous hot extraction method for almost 72h. Solvent from the extract was evaporated by using a rotary evaporator. After rotary evaporation, the removal of remaining amount of solvent was done by following the process of lyophilization commonly known as freeze-drying for 3-4 days. The percentage yield of extract was calculated by using the following formula

$$\% \text{ yield} = \frac{\text{mass of extract} \times 100}{1000}$$

Albino mice were used for testing purpose due to their close relation with human beings. Acute toxicity of the seeds extract was done for 3 days by giving different doses of the testing extract. It was found that seeds extract did not cause serious effects or death in any of the mice. Mice were divided into 3 groups. Group 1 was provided with normal diet and diabetes was not induced in its animals. While in other two groups (labelled group 2 and 3) diabetes was induced by using aqueous solution of Mono-sodium glutamate which was prepared by dissolving 60g of MSG in 1000ml of water. MSG solution was injected through a 1cc syringe into the abdomen of mice. MSG was injected to mice for a week along with fat rich diet. After one week of injecting MSG, glucose levels were checked in mice and it was found that blood glucose levels were raised from normal, Mice with glucose level above 150mg/dl were selected for further treatment. Group 1 received no treatment and was only offered standard diet pellets and freshwater. Glibenclamide 5mg/kg of the dose was also administered to group 2 animals orally with the help of an esophageal catheter for the treatment of diabetes. Group 3 was served with *C.intybus* seeds extract of 500mg/kg concentration and entertained with standard diet pellets and freshwater.

Blood samples were collected from the tail of mice by tail tipping method with the help of a glucometer puncture for consecutive 14 days. After blood collection, the glucose level was checked with the help of a glucometer.

**Results and Discussion**

The ratio of diabetes increasing continuously, particularly diabetes that is inherited is more advanced and this must be treated in the earlier stages otherwise this can be fatal. More appropriate treatment is needed for this disease (Baily, *et al.*, 1986).

There is a wide range of medicinal plants that shows hypoglycemic effect. Many examples are set for the use of medicinal plants for hyperglycemic effects in tradition. According to literature *C. intybus* showed antimicrobial, antiviral, antioxidant, anti-asthmatic, antipyretic, antitumor, antiulcer, anti-inflammatory and anti-diabetic properties, So, it can minimize diabetic constituent and blood glucose level due to its properties.

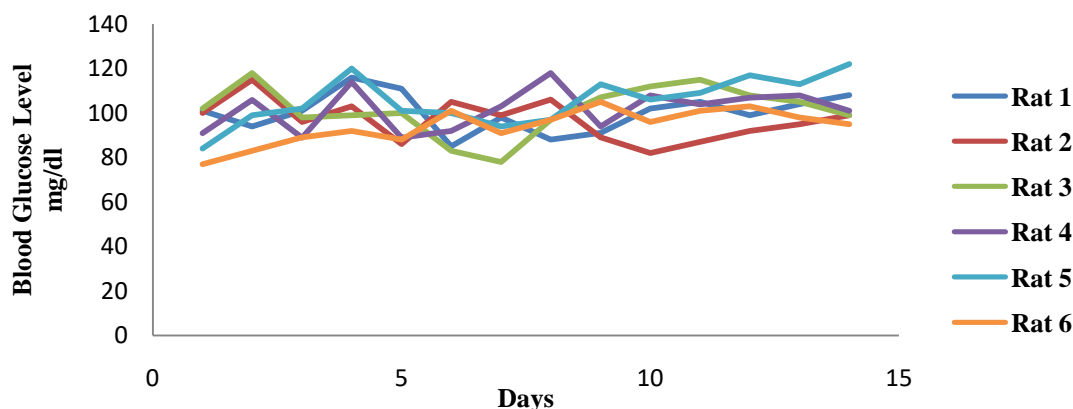
The percentage for *C.intybus* extract was found to be 4.322% with dark brown color. Group 1 is the normal group with no induced diabetes, so it had no specific change in blood glucose level. The glucose level of blood was between 77-120mg/dl (Figure 1).

Group 2 was given a standard dose (Glibenclamide 5mg/kg). It is commonly given for about 14 days to treat diabetes. The Glibenclamide gave a significant decrease in blood glucose level. After treatment of 14 days, the blood glucose level range was 123-195mg/dl (Figure 2).

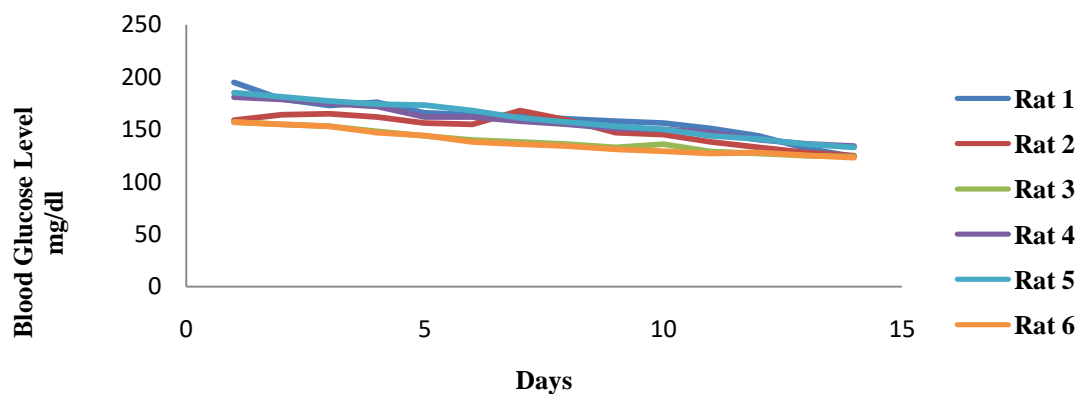
Group 3 was given with *C.intybus* seeds extract of 500mg/kg, the blood glucose level was lowered significantly. It was between 122-185mg/dl. The blood glucose level was 185mg/dl in mice 6 at the start of treatment. It was lowered to 127mg/dl. Mice 4 had lowest blood glucose level 175mg/dl. It was lowered to 122mg/dl. So, it was clear that a 500mg/kg dose of *C. intybus* extract showed antidiabetic activity (Figure 3) (Table 1).

**Table 1. Blood glucose level of mice for 14 days.**

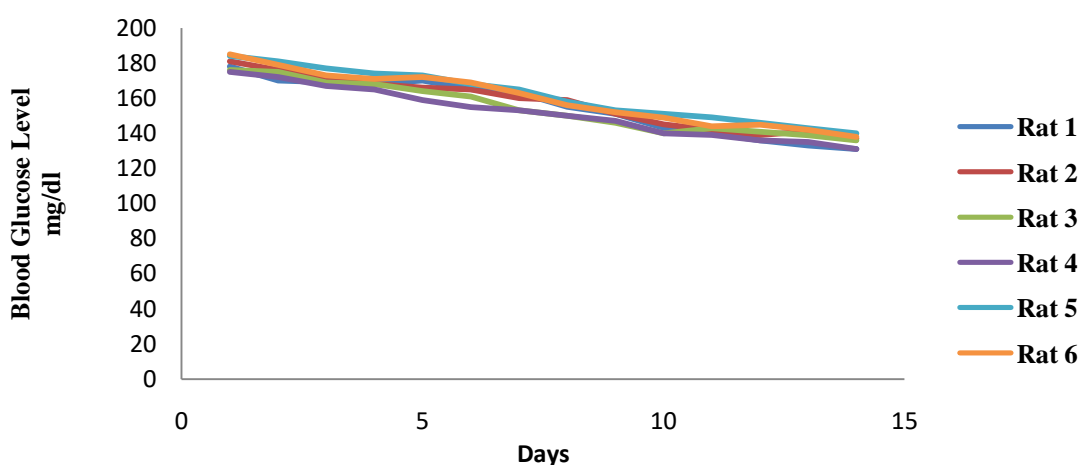
		Blood glucose level (mg/dl)													
Group	Rat	Days													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Group 1 (normal)	1	101	94	101	116	111	85	98	88	91	102	105	99	104	108
	2	100	115	96	103	86	105	99	106	89	82	87	92	95	99
	3	102	118	98	99	100	83	78	97	107	112	115	108	105	99
	4	91	106	89	114	89	92	103	118	94	108	104	107	108	101
	5	84	99	102	120	101	100	94	97	113	106	109	117	113	122
	6	77	83	89	92	88	101	91	97	105	96	101	103	98	95
Group 2 (Glibenclamid)	1	195	179	173	176	166	164	163	160	158	156	151	144	132	124
	2	159	164	165	162	156	155	168	159	147	145	138	133	128	125
	3	157	155	153	148	144	140	138	136	133	136	129	127	125	124
	4	181	179	175	172	167	162	158	155	151	150	147	140	136	134
	5	185	181	177	174	173	168	161	157	153	150	144	141	136	133
	6	157	155	153	147	144	138	136	134	131	129	127	128	125	123
Group 3 (500mg/kg seed extract)	1	178	170	169	168	170	165	163	155	151	143	140	136	133	129
	2	181	176	172	168	166	165	160	159	151	145	142	139	135	131
	3	176	175	169	168	164	161	153	150	146	140	143	141	139	128
	4	175	172	167	165	159	155	153	150	147	140	139	136	135	122
	5	184	181	177	174	173	168	165	158	153	151	149	146	143	126
	6	185	179	173	171	172	169	163	156	152	149	144	145	142	127



**Fig.1. Blood glucose levels (mg/dl) in Group 1 albino mice.**



**Fig.2. Blood glucose levels (mg/dl) in Group 2 albino mice.**



**Fig.3. Blood glucose levels (mg/dl) in Group 3 albino mice.**

## Conclusion

With the use of seed extract of *Cichorium intybus* glucose level of Albino mice was decreased regularly so it was confirmed that this extract can be used to cure diabetes. *C. intybus* is herbal medicine so there is no danger of any side effects which can be caused due to the use of synthetic drugs. With the use of this extract, glucose level can also be controlled. We should encourage the use of these less expensive medicines, with good activity and have no side effects at all.

## Acknowledgment

The Authors of this study are thankful to all coworkers, lab analysts, friends and family members for their efforts, care and support to conduct this research work.

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