DOI: https://doi.org/10.53350/pjmhs22161820

ORIGINAL ARTICLE

Evaluation of Antidiabetic Effect of Silybum marianum and Cichorium intybus extracts

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ABSTRACT

Enormous efforts have recognized the importance of analyzing plant extracts for possible medical applications. In this context, Kasni (C. intybus) and Milk thistle (S. marianum), cultivated in Asia, were used to determine diabetes mellitus control. Ethanol extracts of both plants were used after induction of diabetes mellitus in rabbits (with STZ). Blood glucose was measured by the glucose oxidase method. After treatment of the injured pancreas for 60 days, Kasni and Milk thistle showed a decrease in the elevated levels of blood sugar. The coalesce effect of both plants is more than the individual plants whereas Milk thistle has demonstrated a better control than Kasni. To conclude, C. intybus and S. marianum play a positive role in controlling diabetes.

INTRODUCTION

The phytochemical components are extracted from plants by different methods. These components have great medicinal importance. Our body's metabolism faces a number of beneficial and harmful chemicals. Reactive oxygen species (ROS) are generated in the body during different biochemical reactions.[1].

Its anti-diabetic effects are used to decrease the glycemic index and to prevent diseases related to diabetes. Diabetes affects the organs and produces micro and macrovascular injuries. [2]. Good glycemic control can avoid the metabolic dysfunction leading to any pathological conditions diabetic retinopathy can be avoided by controlling diabetes [3]. These changes are mainly due to microvascular changes affecting the lens and causing opacity [4].

Cichorium intybus having antioxidant effects has also had other beneficial effects on other organs. [5].Therefore there is an increase in oxidative stress in the brain. Cichorium intybus plays a role to prevent this stress [6].

Cichorium intybus, commonly known as Kasni in Pakistan, India, and Iran. It has different

important chemicals such as alkaloids, inulin, among many others. The major component of Cichorium intybus in fresh plants is inulin. Milk thistle (Silybum marianum) Silybum marianum (Silymarin), commonly known as Milk thistle, Silymarin plays a significant role in diabetes due to its antioxidant abilities. Huseini, H. Fallah, et al experimentally performed glycemic control in diabetic patients by silymarin [7]. In the beginning, the diabetic patient's blood glucose value was 188±48 mg/dL, after 4-month treatment of silymarin serum glucose significantly decreased to 133 ± 39 mg/dL. Silymarin administration for four months to type II diabetic patients significantly improved the glycemic profile of patients. It is experimentally proved that silymarin plays a significant role in Alexon induced diabetes in

rabbits. The streptozotocin (STZ) was given to rats in a dose of 50 mg/kg body weight by

intravenous injection, which damages the beta cells of the pancreas and induces diabetes. [8,9].

An anomalous function of alpha and beta cells in panrease can lead to failures in maintaining glycemia. When beta cells are damaged due to any reason the glucose level rises up and leads to diabetes.[10,11].

3. Plants extract

Figure 1: Flower of Silybum marianum (left) and plant of Cichorium intybus (right)

All plants were cleaned up separately with tap water to remove dust and dried after removing dust Subsequently, smaller pieces were ground into a fine powder. The ethanol extract of the plant was obtained by a soaking method with some modifications as described by Imran et al., 2012 [12]. The analytical grade ethanol (99.9%) was used for the extraction of plants. [13]. After the process of extraction, the next step is filtration. The filter papers Whatman No.1 were used. Filtration is used to remove unnecessary material and residue. Filtration is a slow and time-consuming process [14]. The leftover ethanol evaporates at 60 ºC in the incubator leaving behind the crystals in the beaker. These crystals are polar compounds of plants and were stored for use as medicine [9,10].

Figure 2: Ethanol extract of Silybum marianum (left) and Cichorium intybus (right)

4. Animals and treatment protocols: The present study was performed on rabbits. Male rabbits weighing 1-1.5 kg were used. All the rabbits were divided into five subgroups (G1, G2, G3, G4, and G5). Each subgroup consists of 5 rabbits. In this study, diabetes was induced by giving STZ in a dose of 50 mg/kg body weight by intravenous injection. Streptozotocin (STZ) damages the beta cells of the pancreas. Beta cells produce insulin. Hence insulin level is low in the body and glucose level increases in the blood.

The 1st group of rabbits was fed a normal diet. This group serves as a control group.

The 2nd group was administered streptozotocin (50 mg/kg). Streptozotocin damages beta cells of the pancreas and insulin levels are low in the body. STZ induced diabetes in 48 hours after intravenous injection.

The 3rd group of rabbits was treated with streptozotocin (50 mg/kg given by I/V route), milk thistle extract, and milk thistle powder in a dose of 100 mg/kg body weight per day.

The milk thistle extract (50 mg/kg) is given orally to rabbits. The 4th group of rabbits were treated with streptozotocin (50 mg/kg), Kasni extract, and Kasni powder (50 mg/kg) given orally to rabbits. The 5th group of rabbits was administered streptozotocin (50 mg/kg). This group also received extracts of both plants. The Cichorium intybus extract (50 mg/kg) and Silybum marianum extract (50 mg/kg) are separately given to the rabbits daily. The rabbits with damaged pancreas were also treated with powder (100 mg/kg) of both plants daily. After 60 days of treatment with drugs and plant extracts the blood samples were taken from the ear vein of rabbits and glucose level was estimated. The blood sugar (random and fasting) was measured with the glucose oxidase method. [11,12]

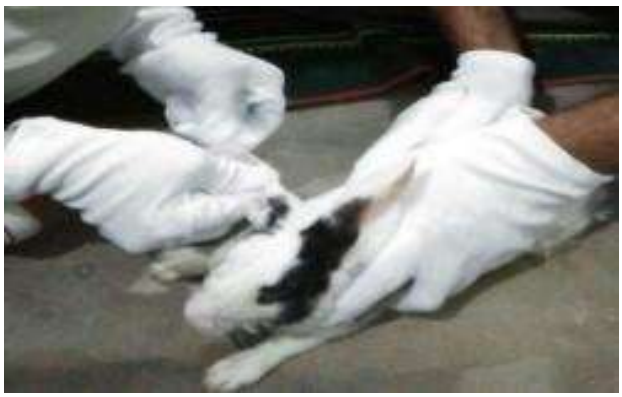

Figure 3: Collection of blood samples

Random sugar test protocol: Glucose was measured by the glucose oxidase method in the biochemistry lab.

Table 1: The values of random blood sugar levels.

Group	G1 Control	G2 (STZ)	G3 (STZ+ MT)	G4 (STZ+K asani)	G5 (STZ+kas ani+MT)
1	113	240	132	143	121
2	120	180	122	130	118
3	108	198	120	147	109
4	98	292	156	183	132
5	102	302	161	190	141

Normal range = (75-140 mg/dL)

Fasting sugar test protocol: Glucose was measured by the glucose oxidase method in the biochemistry lab.

Table 2: The values of fasting blood sugar levels.

Group	G1 Control	G2 (STZ)	G3 (STZ+ MT)	G4 (STZ+K asani)	G5 (STZ+kas ani+MT)
1	77	130	129	132	90
2	80	133	113	119	92
3	71	194	120	137	79
4	82	186	119	140	89
5	85	201	140	153	98

Normal range = (75-140 mg/dL)

4. Effect of plant extract on random blood sugar: The normal value of blood sugar in rabbits in the controls is 75-140 mg/dL. The mean plus standard deviation (M±SD) of the G1 group (control group) was 108.2±8.7. In the G2 group, STZ (streptozotocin) was administered, STZ increased the blood glucose level. The M±SD of the G2 group was 242.4±54.5. It was highly significant (***) as compared to the control group as $p<0.05$. The treatment with C. intybus and S. marianum significantly decreased the value of blood sugar. In the G3 group, the rabbits have treated with STZ and Milk thistle. The M±SD of G3 was

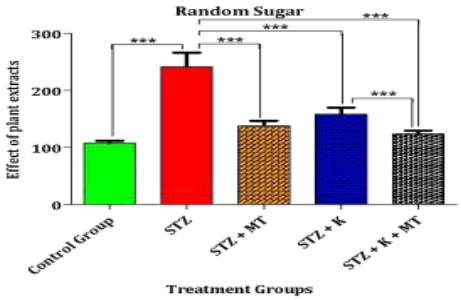

Figure 4: The graph represents the comparison of M±SD values of random sugar of five groups

138.2±19.2 which is close to the control group. It was highly significant (***) as compared to the STZ group because it decreased the value of blood sugar. The G4 group was treated with C. intybus and STZ. The M±SD of the G4 group was 158.6±26.4. It was significant (***) as compared to the G2 group because it decreased the value

of blood sugar. The G5 group is very interesting because extracts of both plants were used. The G5 group was treated with C. intybus, Milk thistle, and STZ. The M±SD of G5 was 124.2±12.5. It was highly significant (***) as compared to the G2 group because these plants decreased the value of blood sugar close to the control group. The P value was < 0.000 which is highly significant as compared to control (p<0.05). The confidence level is (95.0%).

5. Effect of plant extract on fasting blood sugar: The normal blood sugar value of rabbits in control is 75-140 mg/dL. The M±SD of the G1 group (control group) was 79±5.3. In the G2 group, STZ (streptozotocin) was administered, STZ increased the blood level of sugar. The M±SD of the G2 group was 168.8±34.5. It is highly significant (***) as compared to the control group p<0.05. The treatment with C. intybus significantly decreases the value of blood sugar. In the G3 group, the rabbits were treated with STZ and milk thistle. The M±SD of G3 was 124.2±10.5 which is close to the control group. It is highly significant (***) as compared to the STZ group because it decreases the value of blood sugar. The G4 group was treated with C. intybus and STZ. The M±SD of the G4 group was 136.2±12.4. It is significant (***) as compared to the G2 group because it decreased the value of blood sugar. The G5 group is very interesting because extracts of both plants were used. The G5 group was treated with C. intybus, milk thistle, and STZ. The M±SD of G5 was 89.6±6.9. It is highly significant (***) as compared to the G2 group because these plants decrease the value of blood sugar close to the control group. The P value is < 0.000 which is highly significant as compared to controls (p<0.05). The confidence level is (95.0%).

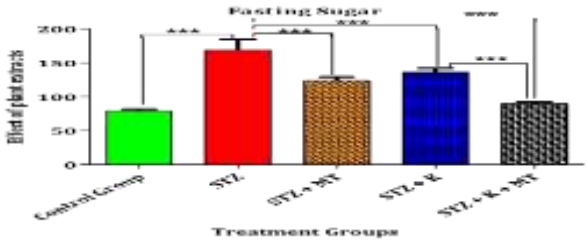

Figure 5: The graph represents the comparison of MSD values of fasting sugar of five groups.

Table 3: Overall M±SD of fasting and random sugar.

MSD	G1 MSD	G2 MSD	G3 MSD	G4 MSD	G5 MSD
	Control	STZ	MT+STZ	K+STZ	K+STZ+MT
Random sugar control 75-140 mg/dL	108.2±8.7	242.4±54.5	138.2±19.2	158.6±26.4	124.2±12.5
Fasting sugar	79±5.3	168.8±34.5	124.2±10.5	136.2±12.4	89.6±6.9

MSD = Mean ± standard deviation; STZ = Streptozotocin; MT = Milk thistle (S. marianum); K = Kasni (C. intybus).

DISCUSSION

The studies on plants for the exploration of their medicinal importance have gained significant attention throughout the world. Much evidence shows the great potential of medicinal plants which have been used traditionally for centuries. Phytochemical components are extracted from plants by different methods. Various plants provide such types of sources of antioxidants. In this study, different compounds with known antioxidant activities were used [15].in our experiments to investigate the beneficial effects of C. intybus and S. marianum on diabetes. Cichorium intybus and Silybum marianum decrease the level of lipid peroxidation and production of oxidative stress. [16]. Glc-6-Pase catalase is an enzyme that plays important role in the last steps of glucose production and in controlling normal blood glucose levels.[17]. There are also studies which shows its antihyperlipidemia effect and there is a significant decrease in the level of serum cholesterol [18]

CONCLUSIONS

Cichorium intybus and Silybum marianum have antioxidant potential which reduces the free radicals. They play a protective role in diabetes. Cichorium intybus has the potential to decrease blood glucose but Silybum marianum has more potential against diabetes than Cichorium intybus.

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