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Investigating the therapeutic potential of aqueous extraction of curry plant (*Murraya koenigi*) leaves supplementation for the regulation of blood glucose level in type 2 diabetes mellitus in female human subjects

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Abstract: Type 2 diabetes mellitus is characterized by hyperglycemia and insulin resistance. It is spreading around the globe like a pandemic. Major factors behind the development of diabetes can be genetics, environmental factors, dietary choices and obesity. Many medicinal plants have anti-diabetic potential. This study has investigated the anti-diabetic effect of curry leaves extract. This study also investigated the chemical characterization of curry leaves. Phytochemicals including saponins, tannins, alkaloids, flavonoids, phenols and glycosides were also investigated. Encapsulated 5mg per kg of the body weight and 10mg per kg of the body weight were given to treatment groups I and II. Random blood sugar, fasting blood sugar, and HbA1c of 45 diabetic female adults were measured on the 0-day and 45th days. All results were analyzed using the two-sample t-test in IBM SPSS Statistics 20. Curry leaves contained moisture (24.1±1.78)%, ash (17.82±2.13)%, nitrogen free extract (36.12±3.52)%, crude protein (8.32±0.83)%, crude fiber (6.98±2.31)% and crude fat (6.87±0.21)%. Mineral analysis showed that magnesium and calcium were major minerals present in curry leaves. Curry leaves extract contained saponins 2.71±0.23, flavonoids 7.84±0.42, tannins 0.91±0.09, glycosides 0.17±0.01, phenols 3.89±0.12, alkaloids 2.01±0.87. These phytochemicals were expressed in mg/100 g of the sample. Curry leaf extract showed a significant ($p<0.05$) reduction in fasting blood sugar, random blood sugar and glycated hemoglobin in both treatment groups.

Keywords: Diabetes, insulin, supplementation, alkaloids, phytochemicals, tannins, flavonoids.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic condition of dysregulated glucose metabolism. It is a condition characterized by the development of insulin resistance and impaired insulin secretion of the pancreas. Conditions like hyperglycemia and ketoacidosis are often observed in patients with T2DM (Abdelsamia *et al*., 2019). It is spreading like a pandemic around the globe. The number of people suffering from type 2 diabetes mellitus has doubled. There are many reasons behind the ever-increasing prevalence of diabetes are genetics, environmental factors, dietary habits and obesity (L. Chen *et al*., 2012). Diabetes needs management as it also increases the risk of associated complications like nephropathy, neuropathy, muscle loss, kidney failure and cardiac issues as well. Management of diabetes is very important in hospital care protocols. Various drug therapies, insulin therapies and other medications are used to regulate impaired glucose metabolism and hyperglycemia in type 2 diabetes mellitus (Clement *et al*., 2004). Apart from these pharmaceutical approaches to manage diabetes, many naturally existing plants, herbs, and spices are used to manage the signs and symptoms of diabetes. These herbs and plants possess bioactive compounds which help in managing insulin resistance and hyperglycemia (Rizvi & Mishra, 2013). *Murraya Koegini* i.e., curry leaves are often used for the management of diabetes by the population. Curry leaves are used in foods for natural flavoring which also adds aroma to the food. Curry leaves are also used in the cosmetics and pharmaceutical industry. Curry leaves contain many active compounds including glycosides, flavonoids and carbazole alkaloids. These active compounds help in the regulation of hyperglycemia and insulin resistance (Al-Ani *et al*., 2017). The normal value of random and fasting blood sugar levels is measured to check diabetes. To

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investigate insulin resistance HbA1c is measured in the blood (Bnouham *et al*., 2002). This study was focused on the determination therapeutic potential of phytochemicals which regulate blood glucose levels and HbA1c in diabetes patients. This study has also investigated the chemical composition of curry leaf extract including proximate analysis, mineral determination of curry leaves, and phytochemicals present in curry leaves extract.

MATERIALS AND METHODS

Collection of raw material and preparation of curry leaves powder

Curry leaves were purchased from the local market of Karachi. For the identification of curry leaves, assistance was taken from Botanists of University of Karachi. Curry leaves that were already free from debris and dirt. However, curry leaves were removed from the packaging and washed first with tap water and then with distilled water. After washing, the leaves were dried on a forced conventional tray at 60^{o} for 4 hours. Then leaves were ground to refine powder and stored in air-tight jars (Y. Chen *et al*., 2015).

Preparation of curry leaves extract

After drying, 10 g of curry leaves powder was mixed with 100 ml of deionized water and heated in a water bath for 1 hour at 90^{o} After 1hour, extraction was passed through muslin cloth and stored in amber color bottles. 10 ml and 15 ml of curry leaves extract was mixed with carrier agents including 10% maltodextrin, 2% gum arabica, 0.5% xanthan and 5% soluble starch. This mixture was retained overnight for rehydration and then was encapsulated in gelatin capsules by spray drying doses mentioned in treatment plan (table 1) (Sablania *et al*., 2018). Gelatin capsules found to be safe for human consumption (Gullapalli, 2010).

Chemical characterization of curry leaves extract

Proximate Analysis of Curry Leaves Powder

Curry leaves powder was analyzed for the following proximate profile including moisture content, ash, carbohydrates, crude protein, crude fiber, and nitrogen-free extract (NFE) according to the AOAC method (Matsuo *et al*., 2019).

Minerals analysis of curry leaves extract

The following minerals zinc, magnesium, iron, phosphorus, potassium, sodium, and copper were analyzed in curry leaf extract by using atomic absorption spectrometry (Hernández *et al*., 2005).

Phytochemical composition of curry leaves extract

About 2-3 gm sample of curry leaves extract was defatted using diethyl ether. The harbone method was used to evaluate the amount of various phytochemicals including flavonoids, alkaloids, glycosides, phenols, saponins and tannins (M'hiri *et al*., 2015).

Investigation of antihyperglycemic potential of curry leaves extract

Selection of Subjects

After taking informed and unpressurized consent, 45 diabetic female subjects of age 35-60 years, who were taking no antidiabetic medications were randomly selected from the population.

Exclusion criteria

Following subjects were not included in the study:

- Female diabetic patients with other complications like ischemic heart disease.
- Planning to be pregnant, pregnant, and lactating women.
- Those females who were taking any medications to control blood sugar levels.

Inclusion criteria

All diabetic female subjects went through blood biochemical analysis by checking their random blood sugar (RBS), fasting blood sugar (FBS) and glycated hemoglobin (HbA1c) at the start of the study.

Study duration and study design

The study was conducted for 45 days and it was a controlled randomized trial (Bhide *et al*., 2018).

Treatment groups and treatment plan

Female subjects were divided into three groups of 15 subjects in each group. The control group received no treatment. Treatment group-I and treatment group 2 received encapsulated curry leaves extract in doses of 5mg/kg of body weight and 10 mg/kg of the body weight shown in table 1.

STATISTICAL ANALYSIS

Descriptive statistical analysis using the two-sample t-test under a Completely Randomized Design (CRD) was carried out to investigate the level of significance ($p<0.05$). Results were shown as mean± S.D. All statistical analyses were done with IBM SPSS Statistics 20.

Ethical approval

Ethical approval was taken from the biosafety committee, Agriculture University Faisalabad Ref#Ec-171.

RESULTS

This research study was designed to determine chemical composition, phytochemical content, mineral contents, and changes in blood glucose levels by consuming curry leaf extract. Parameters including random blood sugar (RBS), fasting blood sugar (FBS) and glycated hemoglobin (HbA1c) were measured on the 0-day and 45^{th} day.

Proximate composition of curry leaves

The encapsulated curry leaves extract was investigated for varied characterizations, such as moisture, ash, crude fiber, crude proteins, fat and nitrogen-free extract (NFE) depicted in table 2. Curry leaves contain moisture (24.1±1.78)%, ash (17.82±2.13)%, nitrogen free extract (36.12±3.52)%, crude protein (8.32±0.83)%, crude fiber (6.98±2.31)% and crude fat (6.87±0.21)%.

Mineral analysis of curry leaves extract

In the current study, curry leaves extract was also analyzed for the determination of mineral content including calcium, potassium, zinc, magnesium and sodium depicted in table 3. Curry leaves contain magnesium in the highest amount. Findings have shown that curry leaves contain magnesium (572.21±12.98), calcium (2220.23±10.87), zinc (2.51±0.001), potassium (1198±13.86), and sodium (47.21±3.17). These findings were shown in mg per 100g in fig. 1.

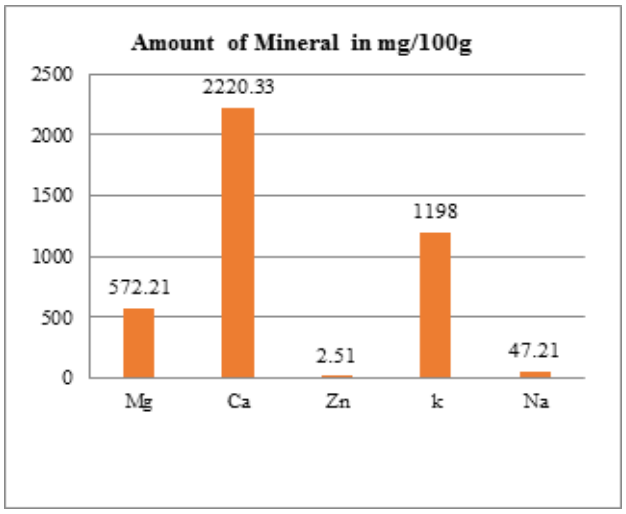

Fig. 1: Mineral composition in dried curry leaves

Phytochemical characterization of curry leaves extract

Curry leaves powder was analyzed for total phenolic content (TPC) and (TFC) analysis. It carried significant amounts of various phytochemicals shown in table 3. These phytochemicals were expressed in mg/100g of the sample.

Table 1: Treatment groups and treatment plan

Treatment Groups	Title	Treatment
T_0	The control group	No treatment
T_1	Treatment group-I	5mg/kg of the body weight of curry leaf extract
T_2	Treatment group II	10mg/kg of the body weight of curry leaf extract

Table 2: Mean ± S.D for proximate % composition of curry leaves powder

Proximate analysis	Composition (%)
Moisture	24.1±1.78
Ash	17.82±2.13
Nitrogen Free Extract	36.12±3.52
Crude protein	8.32±0.83
Crude Fat	6.98±2.31
Crude fiber	6.87±0.21

Table 3: Mean ± S.D for phytochemical analysis of curry leaves extract

Phytochemical	Amount in mg/100g
Saponins	2.71±0.23
Flavonoids	7.84±0.42
Tannins	0.91±0.09
Glycosides	0.17±0.01
Phenols	3.89±0.12
Alkaloids	2.01±0.87

Investigation of therapeutic effects of curry leaf extract against diabetes

The objective of this study was to investigate the antidiabetic effect of curry leaf extract in female diabetic subjects. Participants of the control group (T_0) were given no treatment while participants of treatment group-I (T_1) and treatment group-II (T_2) were given encapsulated curry leaf extract in doses of 5 mg and 10 mg per kg of the body weight respectively for 45 days.

Random blood sugar levels of female subjects

The investigation showed a significant ($p<0.05$) reduction in random blood sugar levels in both treatment groups. Group-I and group II showed a reduction in random blood sugar levels. However, the control group showed no significant change in random blood sugar levels on the 45th day as compared to the 0-day.

Table 4: Mean±S.D for random IV. blood sugar level of female subjects in g/dl

Duration	T_O	T_1	T_2
0 day	283.0± 11.27	279.23± 13.85	292.32± 15.67
45th day	285.70± 10.87	262.12± 18.74*	244.34± 17.85*

T_0 = No Treatment, T_1=5mg/kg of body weight of curry leaves extract, T_2= 10mg/kg of body weight of curry leaves extract. All results were significant at $p<0.05$.

Fasting blood sugar level of female subjects

Both experimental groups showed significant ($p<0.05$) reduction in fasting blood glucose levels in T_1 and T_2 respectively. Curry leaf extract reduced elevated fasting

glucose levels. FBS remained almost unchanged in the control group.

Table 5: Mean±S.D for fasting blood sugar level of female subjects

Duration	T_O	T_1	T_2
0 day	169.43±13.84	189.20± 16.87	176.20± 11.87
45th day	171.70± 11.95	174.30± 17.96*	154.40± 14.85*

T_0 = No Treatment, T_1=5mg/kg of body weight of curry leaves extract, T_2= 10mg/kg of body weight of curry leaves extract. All results were significant at $p<0.05$.

HbA1c levels of female subjects

The investigation showed a significant ($p<0.05$) decrease in HbA1c levels in both treatment groups in response to curry leaf extract. Group-I and group-II showed a reduction in HbA1c. However, the control group showed an increase in random blood sugar levels on the 45th day as compared to the 0-day.

Table 6: Mean±S.D for HbA1c level of female subjects

Duration	T_O	T_1	T_2
0 day	8.94±2.10	8.85±0.31	8.73±1.32
45th day	8.97±1.42	7.62±0.52*	6.84±1.12*

T_0 = No Treatment, T_1=5mg/kg of body weight of curry leaves extract, T_2= 10mg/kg of body weight of curry leaves extract. All results were significant at $p<0.05$.

DISCUSSIONS

All these findings showed almost similar values to that of the previous literature. Findings of proximate value help the researchers to determine the chemical characterization and quality of the raw material used. The proximate analysis contributes to the major nutritional profile of any item. Minor differences in the values can be due to differences in the variety of curry leaves used and environmental conditions (Parnami & Varma, 2019). In this study, amounts of Ca, Mg, Zn, K and Na were also determined. These findings were similar to the findings of previous studies (Parnami & Varma, 2019). Curry leaves contain a generous amount of magnesium that also plays a role in the regulation of insulin functioning in the body. The findings of the study have shown that curry leaf extract contains significant amounts of saponins, phenols, alkaloids, flavonoids, tannins and glycosides. All these findings were like previous findings with minor differences. These minor differences might be due to variations in the curry leaves used in studies or might be due to environmental conditions. Findings have shown that curry leaves contain significant amounts of flavonoids, phenols, alkaloids and saponins. All these phytochemicals have therapeutic potential against diabetes (Igara *et al*., 2016).

The findings of the study revealed that curry leaves extract decreased the level of random blood sugar, fasting blood sugar, and HbA1c in both treatment groups. These findings were also observed in another study conducted on diabetic rats in which an aqueous extract of curry leaves not only reduced blood glucose levels but also reduced elevated HbA1c in diabetic rats. The study also investigated the antioxidant potential of curry leaf extract. The study reported that curry leaf extract has the potential to reduce oxidative stress-induced damage to islets of Langerhans (Al-Ani *et al*., 2017). Another study also investigated the hypoglycemic effect of curry leaves powder supplementation on diabetics. The study reported the same findings that curry leaves powder reduce elevated blood glucose level when given to patients for thirty days. The study also reported that curry leaves powder improves insulin action and reduces insulin resistance which is also observed in the understudy research (Kirupa & Kavitha., 2013).

Another study showed similar results when curry leaves were used against diabetes in rats which reported the hypoglycemic potential of curry leaves. The trial was continued for seven days and moderately diabetic rats showed improvement in blood glucose levels (Yadav *et al*., 2002). Similar findings were also observed in another study in which antihyperglycemic effect of curry leaves extract was studied alone and in combination with extract of *Vitis vinifera*. That study reported that the combination showed better hypoglycemic effect than curry leaves extract only. (Palwankar *et al*., 2020). Similar findings were also reported in a study that investigated the phytochemicals in curry leaves along with phytochemicals in garlic. Phytochemicals were reported to have antidiabetic effect (Bhowmik *et al*., 2021).

CONCLUSION

Curry leaves are widely used in spices and natural flavoring. They show antidiabetic, antihyperglycemic and antioxidative properties along with an important nutritional profile. Curry leaf extract also contained generous amounts of phytochemicals including saponins, flavonoids, alkaloids, glycosides and tannins. All these phytochemicals contribute to incorporating the antioxidant properties of curry leaf extract. The study has reported a reduction in random blood sugar level, fasting blood sugar level and HbA1c in response to the two different doses of curry leaves extract when consumed in encapsulated form. Phytochemicals in curry leaves are helpful in reducing insulin resistance by scavenging the free radicals in the body. Thus, curry leaves extract is beneficial for patients with hyperglycemia and diabetes.

Curry leaves are also beneficial in reducing oxidative stress and provide important minerals to the body that are helpful in regulating the insulin function.

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