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Investigating the nutraceutical potential of apple peel extract supplementation for regulating the glucose metabolism in hyperlipidemic Female human subjects

Zaheen Aysha Iftikhar[1], Urwah Shahid[2], Madiha Farooq[3], Haleema Kamila[4], Nusrat Ali[5], Maryam Mastoor[6], Saeedah Musaed Almutairi[7], Rabab Ahmed Rasheed[8] and Tse-Wei Chen[9]

[1]BHU Budh Ghulam, Vehari, Pakistan
[2]BHU Meeray Shah Tehsil Sadiqabad, Rahim Yar Khan, Pakistan
[3]DHQ Teaching Hospital, Faisalabad, Pakistan
[4]Kuwait Teaching Hospital, Peshawar Medical College, Peshawar, Pakistan
[5]Department of Biochemistry, HBS Medical and Dental College, Islamabad, Pakistan
[6]Department of Biochemistry, Amna Inayat Medical College, Lahore, Pakistan
[7]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia
[8]Histology & Cell Biology Department, Faculty of Medicine, King Salman International University, South Sinai, Egypt
[9]Department of Materials, Imperial College London, London, United Kingdom

Abstract: Hyperglycemia is a condition often observed in diabetics, dyslipidemia and obese. It is a major factor behind the development of diabetes and the reasons can be genetics, environmental factors, dietary choices, and obesity. Many medicinal plants have anti-diabetic potential. This study investigated the anti-hyperglycemic effect of apple peel extract. This study also investigated the chemical characterization of apple peel. Phytochemicals including total phenolics and flavonoids were determined. Encapsulated 350mg/day was given to treatment groups. Random blood sugar, fasting blood sugar and HbA1c of 45 diabetic female adults was measured on the 0-day and 45th day. Results showed that apple peel contained moisture (14.71±3.57)%, ash (17.82±2.13)%, nitrogen free extract (32.12±3.52)%, crude protein (6.89±0.83)%, crude fiber (19.17±0.21)% and crude fat (9.91±2.31)%. Findings showed that apple peel contains magnesium (6.61±1.088), calcium (8.17±0.32), zinc (14.08±1.21) and potassium (67.21±1.86). These findings were shown in mg in kg. Apple peel extract contained total phenolic content (TPC) of 8.14±1.07 and total phenolic content (TFC) of 4.89±1.81. Apple peel extract showed a significant reduction in all blood parameters of hyperglycemia. All results were significant at $p<0.05$.

Keywords: Apple, apple peel extract, phenolics content, proximate determination, phytochemical analysis, hyperglycemia, random blood sugar.

INTRODUCTION

Epidemiological studies have explained the presence of a strong relationship between metabolic diseases and diet. Metabolic conditions like hyperlipidemia, hyperglycemia, diabetes and obesity are the manifestation of poor dietary choices which often lead to the development of coronary heart diseases and other chronic conditions. A person who consumes more of a balanced diet is less likely to develop all these disorders (Vafa *et al*., 2011). Hyperglycemia, a state of elevated serum glucose level is present in diabetics and obese. Populations with hyperlipidemia often show elevated serum glucose levels too. This elevation is the manifestation of impaired glucose metabolism. Hyperglycemia in the population with hyperlipidemia is an important risk factor for cardiovascular disorders (Davies *et al*., 2018). Hyperglycemia management is crucial as it also increases the risk of associated complications like nephropathy, neuropathy, muscle loss, kidney failure and other issues. Management of hyperglycemia is very important in hospital care protocols. Various drug therapies, insulin therapies and other medications are used to regulate impaired glucose metabolism and hyperglycemia (Clement *et al*., 2004).

Fruits and vegetables are important food groups that not only provide energy but also vital minerals, vitamins, and phytochemicals. These phytochemicals have some medicinal properties. Apple is among the most popular fruits globally and it is grown on a larger scale. It is of many kinds and sizes. Apple has wide food applications and many food products contain apples (Lyu *et al*., 2020). Food waste like peel, pulp and seeds are of great nutraceutical importance as they contain bioactive compounds of medicinal importance. Apple peel is one of the largest produced food wastes both at the domestic and industrial scale. Apple peel is of great nutraceutical and medicinal importance as it contains catechin, epicatechin, quercetin, fiber and chlorogenic acid. Apple has a

*Corresponding author: e-mail: meryummastoor@hotmail.com

tremendous ability to regulate blood glucose levels. Apple peel contains a generous amount of fiber which ensures a gradual rise in blood sugar levels and maintains satiety. Apple, apart from this, has other health benefits too like regulating dyslipidemia, heart health, and preventing oxidative stress (Boyer & Liu, 2004). This study has investigated the medicinal properties of apple peel supplementation against hyperglycemia. Different parameters were measured in the subject. The normal value of random and fasting blood sugar levels is measured to check hyperglycemia. To investigate insulin resistance HbA1c is measured in the blood (Bnouham *et al*., 2002). The objective of the study was to investigate the therapeutic effect of apple peel on random blood sugar, fasting blood sugar and HbA1c. This study also found the chemical composition of apple peel including proximate analysis, mineral determination and phytochemical contents.

MATERIALS AND METHODS

Collection of raw material and preparation of apple peel powder

Red apple was purchased from the local market of Karachi. For the identification of apples, assistance was taken from a Botanist of University of Karachi. Apples were already free from debris and dirt. However, apples were washed first with tap water and then with distilled water. After washing, the peel of the apple was removed and dried on a forced conventional tray at 60^{o} for 4 hours. Then peel was ground to refine powder and stored in air-tight jars (Chen *et al*., 2015). We have selected the patients with hyperlipidemia because the patients with hypertriglyceridemia and and LDL not only the consequences but also the cause of disturb metabolism because the metabolism of glucose fatty acids and cholesterol are often intertwined for example glucose CAn be converted to fatty acids and cholesterol through de Novo lipid biosynthesis pathway. so we selected the patients of diabetes with hyperlipidemia which is the major cause of irregular glucose metabolism

Preparation of apple peel extract

10g of apple peel powder was mixed with 100ml of deionized water and heated in a water bath for 1 hour at 90^{o}. After 1 hour, extraction was passed through muslin cloth and stored in dark color bottles. 350ml of apple peel extract was mixed with carrier agent 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 were found to be safe for human consumption (Touchette & Cox, 2022).

Chemical characterization of apple peel

Proximate analysis of apple peel

Apple peel 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 apple peel

The following minerals zinc, magnesium, iron, potassium, sodium, and calcium were analyzed in apple peel extract by using atomic absorption spectrometry (Hernández *et al*., 2005).

Phytochemical composition of apple peel extract

About 2-3 gram samples of apple peel extract were defatted using diethyl ether. The harbone method was used to evaluate the number of various phytochemicals and flavonoids that were measured in mg gallic acid equivalent and catechin equivalent respectively (M'hiri *et al*., 2015).

Investigation of antihyperglycemic potential of apple peel extract

Selection of subjects

After taking informed and unpressurized consent, 30 diabetic female employees of age 35-60 years were randomly selected from a private company.

Exclusion criteria

Females taking antidiabetic medication

Females' diabetic patients with other chronic diseases

Pregnant and lactating women

Inclusion criteria

All diabetic female subjects with abnormal lipid profile 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 and guidelines were taken from literature (Zabor *et al*., 2020).

Treatment groups and treatment plan

Female subjects were divided into two groups of 15 subjects in each group. The control group was given no treatment. The treatment group or intervention group was given a dose of apple peel extract of 350 mg/day.

Ethical approval

Ethical approval was taken from the biosafety committee Agriculture University Faisalabad Ref#Ec-189.

STATISTICAL ANALYSIS

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

RESULTS

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

Table 1: Treatment groups and treatment plan

Treatment Groups	Title	Treatment
T_0	Control group	No treatment
T_1	Treatment group-I	350 mg/day of apple peel extract

Table 2: Mean ± S.D for proximate % composition of apple peel

Proximate analysis	Composition (%)
Moisture	14.71±3.57
Ash	17.82±2.13
Nitrogen Free Extract	32.12±3.52
Crude protein	6.89±0.83
Crude Fat	9.91±2.31
Crude fiber	19.17±0.21

Table 3: Mean ± S.D for phytochemical analysis of apple peel extract

Phytochemical	Amount
Total Phenolic (mg GAE/g)	8.14±1.07
Total Flavonoids (mg CE/g)	4.89±1.81

Proximate composition of apple peel

The encapsulated apple peel extract was investigated for varied characterizations, such as moisture, ash, crude fiber, crude proteins, fat and nitrogen-free extract (NFE) depicted in table 2.

Mineral analysis of apple peel extract

In the current study, apple peel was also analyzed for the determination of mineral content including calcium, potassium, zinc, magnesium and iron depicted in fig. 1. Apple peel contained potassium in the highest amount.

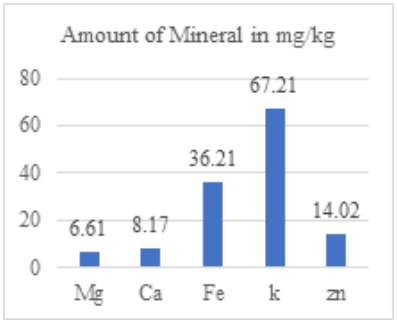

Fig. 1: Mineral analyses.

Phytochemical characterization of apple peel extract

Apple peel extract was analyzed for total phenolic content (TPC) and (TFC) analysis. It carried significant amounts of various phytochemicals shown in table 3.

Investigation of medicinal effect of apple peel extract against diabetes

The objective of this study was to investigate the antidiabetic effect of apple peel extract in female diabetic subjects. Participants of the control group (T_0) were given no treatment while participants of the treatment group (T_1) were given 350mg of apple peel extract per day for 45 days.

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

Duration	T_O	T_1
0 day	287.0±11.27	285.23±11.28
45th day	287.70±10.87	262.12±13.74*

T_0 = No Treatment, T_1= 350 mg per day of apple peel extract. All results are taken significant at $p<0.05$.

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

Duration	T_O	T_1
0 day	169.43±13.84	189.20±16.87
45th day	171.70±11.95	174.30±17.96*

T_0 = No Treatment, T_1= 350 mg per day of apple peel extract. All results are taken significant at $p<0.05$.

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

Duration	T_O	T_1
0 day	8.61±1.87	8.72±0.43
45th day	8.87±1.56	7.24±0.35*

T_0 = No Treatment, T_1= 350 mg per day of apple peel extract. All results are taken significant at $p<0.05$.

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. The treatment group 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.

Fasting blood sugar level of female subjects

Treatment group showed a significant ($p<0.05$) reduction in fasting blood glucose levels. Apple peel extract reduced elevated fasting glucose levels. FBS almost remained unchanged in the control group.

HbA1c levels of female subjects

The investigation showed a significant ($p<0.05$) decrease in HbA1c levels in both treatment groups in response to apple peel extract. The treatment group 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.

DISCUSSIONS

The study was designed to investigate one of the major complications observed in hyperlipidemia i.e., impaired glucose metabolism and insulin resistance. Previous literature had discussed the effect of apples in diabetics but not irregular glucose metabolism in hyperlipidemia patients. In the study, proximate analysis was performed to determine the nutritional profile and quality of the raw material used. It was also helpful in determining the quantity of fiber which also has nutraceutical properties. Proximate analysis values for apple peel were shown in table 2. These findings were similar to previous literature with minor differences. These slight differences are due to fluctuations in environmental conditions (Akpabio *et al*., 2012). The results showed the amount of iron, magnesium, calcium, potassium, and zinc in mg/kg of apple peel. Potassium was present in the highest amount among these minerals. All these minerals possessed vital physiological functions. The findings of these studies were similar to the findings of previous literature (Henríquez *et al*., 2010). Apple peel contained significant amounts of phytochemicals. The total phenolic content and total phenolic content in apples were represented in table 3. These findings were also observed in previous studies that investigated the phenolic content in apple and apple by-products with slight changes (Massini *et al*., 2013). Apple peel extract reduced all random blood sugar, fasting blood sugar, and HbA1c. The presence of significant nutritional composition helped in regulating insulin action, and phytochemicals played a role in reducing elevated blood sugar levels. Apple peels contain quercetin, catechin, epicatechin and many other phytochemicals. These phytochemicals reduced oxidative stress thus decreasing insulin resistance and HbA1c. These findings were supported by previous findings but the study was not conducted in hyperlipidemic patients (Henríquez *et al*., 2020). Another study reported that apple seeds extract showed a reduction in lipid profile when fortified in cereals but these patients were not hyperlipidemic (Fathy & Drees, 2015). A study that addressed the glucose metabolism regulating effect in induced diabetes in rats reported apple peel improved the glucose metabolism. However, the study did not mention that either rats were hyperlipidemic or not (Kamdi *et al*., 2021).

CONCLUSION

Apple is a widely consumed fruit that is grown worldwide and has widespread food applications. It is used in fruits, juices, jams, jellies, and cakes. Apple produces many products including seeds, peel and pulp. These by-products of apples are extremely useful due to the presence of bioactive compounds. These bioactive compounds possess tremendous health benefits. Apple peel extract was investigated against impaired glucose metabolism in hyperlipidemia patients. The study reported regulation of glucose metabolism by maintaining random blood sugar, fasting blood sugar and HbA1c to a reduced level than that of before apple peel extract consumption. The study reported the significance of apple peel extract in the management of impaired glucose metabolism in patients of hyperlipidemia.

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