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Hyperglycemic Activity of *Chenopodium Quinoa* in Diabetic Rats and its Potential Health Benefits – Functional Superfood for Today's World

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ABSTRACT

Introduction: Quinoa is a crop developed regularly as an oat feast. Concerning the most fundamental supplements, it is viewed as the world's perhaps the most popular wellbeing nourishments. Quinoa has over the top nutritive esteem and can acquire food security around the world. The 2013th year was considered as "the international year of the quinoa".

Objective: To evaluate the Phytochemicals, Minerals and Vitamins content, hyperglycemic activity of the quinoa seed powder (QSP) and their effect on diabetic rats.

Methods: Major phytoconstituents in the quinoa seed powder were tested according to standard methods. Minerals and Vitamins were analysed with an atomic absorption spectrophotometer. The antidiabetic effect was evaluated in streptozotocin-induced diabetic rats following oral administration of probiotics and different concentration of quinoa seed powder in.

Results: A large number of minerals found in quinoa seeds powder had the content of Magnesium, Zinc, Chromium, Manganese, and Vanadium. Quinoa seed powder administered up to 28 days (150, 250 and 350 mg/kg) significantly improve the alterations in blood glucose levels in diabetic rats and no histopathological changes were observed.

Conclusion: Incorporation of quinoa in our diet not only increases the nutritive value alternatively will also minimize the risk of various health illness like cardiovascular diseases, type 2 diabetes, etc. Use of quinoa represents a promising region of research as its use in our daily diet can enhance the consumption of certain essential nutrients and phytochemicals which caters important health benefits.

Key Words: Diabetes, Quinoa Seed Powder (QSP), Phytochemicals, Vitamin and Minerals, Histopathology, Probiotics

INTRODUCTION

Diabetes mellitus (DM) is in all likelihood one of the oldest ailments recognised to man. It was once first pronounced in Egyptian manuscript about 3000 years in the past.¹ In 1936, the difference between kind 1 and kind two DM was once definitely made. Type two DM used to be first described as an issue of metabolic syndrome in 1988.² Type 2 DM (formerly recognized as non-insulin dependent DM) is the most frequent form of Diabetes Mellitus characterized by hyperglycemia, insulin resistance, and relative insulin deficiency.³ Type 2 Diabetes Mellitus Outcomes from the interaction between genetic, environmental and behavioural risk factors.⁴ It is estimated that 366 million human beings had DM in 2011; through 2030 this would have risen to 552 million. The number of human beings with type 2 DM is increas-

ing in every country with 80% of human beings with DM residing in low- and middle-income countries. DM caused 4.6 million deaths in 2011. It is estimated that 439 million humans would have kind two DM employing the year 2030.⁵ The incidence of kind two DM varies drastically from one geographical vicinity to the different result of environmental and way of life danger factors. People residing with type 2 DM are more susceptible to various types of short- and long-term complications, which frequently lead to their premature death. This tendency of increased morbidity and mortality is seen in patients with type 2 DM due to the fact of the commonness of this type of DM, its insidious onset and late recognition, particularly in resource-poor developing countries.⁶

Quinoa, a pseudo-cereal, is recognized as one of the world's healthiest foods due to its excessive nutritional value along

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with its potential to cater to various health benefits. Being an accurate source of whole protein (contains all the nine crucial amino acids), unsaturated fatty acids, minerals, vitamins, fibre and antioxidants, it is considered as “superfood”. Quinoa additionally assists with decreasing the danger of different sicknesses like cardiovascular infections, type-2 diabetes, some malignant growth, over the top pulse, heftiness and is likewise a decent choice for people who are hypersensitive to certain nutrition classes. Its biodiversity and functionally to sustain in unfavourable adverse climatic conditions make it a perfect crop to cultivate worldwide particularly in below developing countries of Asia and Africa, where food production is threatened by global climatic changes. Hence, the existing world demands to increase the awareness regarding the various functional benefits of quinoa to fight one of the world’s major crises, that is, starvation and malnutrition.⁷

Quinoa is a species of the goosefoot genus. It’s a crop grown particularly for its edible seeds. Being high in various essential nutrients, it is considered as world’s one of the most popular health foods. The Food and Agricultural Organization of the United Nations (FAO) formally declared the yr 2013 as “The International Year of The Quinoa”. FAO declared quinoa as a portion of food with excessive nutritive value, widespread biodiversity and as a food which can have an important role to play in the achievement of food security worldwide.⁸ Being exceptionally nutritious, quinoa additionally imparts various health benefits which makes it an excellent example of ‘functional food’ as suggested.

Quinoa seeds (*Chenopodium quinoa*) contain a significant number of phytochemicals including flavonoids, phenolic acids, squalene, phytosterol, saponins, fat-soluble vitamins, fatty acids, trace elements and other compounds. The eating regimen of individuals needs numerous supplements, especially some significant minerals like magnesium, potassium, zinc and iron. The absence of iron is perhaps the most incessant nourishment insufficiencies. It keeps our red platelets solid and conveys oxygen starting with one then onto the next cell and builds mind works alongside another significant capacity in our body. Dietary minerals are essentially chemical factors that play a functional role in regulating electrolyte balance, glucose homeostasis, the transmission of nerve impulses and enzyme cofactors in the body. Calcium, magnesium and potassium in quinoa are found in sufficient quantities and bioavailable forms necessary for maintaining a balanced human diet.⁹

Quinoa is also a good source of B nutritional nutrients riboflavin and folic corrosive. Riboflavin improves energy digestion inside mind and muscle cells, and folic corrosive assumes an essential part for legitimate cerebrum work and is significant for acceptable mental and enthusiastic wellbeing. It is a significant nutrient for pregnant ladies as it brings down the danger of unbiased birth surrenders. Quinoa ad-

ditionally includes a significant amount of vitamin E, which acts as an antioxidant, even though the quantity declines after processing and cooking.¹⁰ Low level of vitamin E are associated with an increased incidence of diabetes and some research suggests that people with diabetes have decreased level of antioxidants.

Our study aimed to evaluate the hyperglycemic activity and histopathological changes that occur in diabetic albino rats fed on different concentration of quinoa seed powder. Besides, the willpower of the dietary properties, chemical composition, phenolic content material in quinoa seed powder.

MATERIALS AND METHODS

Quinoa seeds (*Chenopodium quinoa*) was obtained from the online supermarket. The dry seeds were powdered and packaged in moisture-proof containers and stored in a freezer. They were conditioned at room temperature before use.

Phytochemical screening

Major phytoconstituents in the quinoa seed powder such as alkaloids, saponins, tannin, steroids, flavonoids, glycosides, terpenoids and anthraquinone were tested according to standard methods.¹¹⁻¹³

Test for alkaloids

A total of 0.5 g quinoa seed powder was mixed with methanol containing 1% HCl and then boiled and filtered. A total of 2 ml of 10% NH₃ and 5 ml of chloroform was added to 5 ml of the filtrates and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 2 ml of acetic acid, and Mayer’s reagent was added. The formation of cream (with Mayer’s reagent) or presence of turbidity used to be viewed as the presence of alkaloids.

Test for saponins

Two grams of quinoa seed powder was boiled in 20 ml of water in a water bath and filtered. A total of five ml of the filtrates were mixed with three ml distilled water in a test tube and shaken vigorously. Frothing, which persisted on warming, was considered preliminary evidence for the presence of saponins. A few drops of olive oil had been delivered to the extract and shaken vigorously. The appearance of the formation of soluble emulsion in the extracts was indicative for the presence of saponins.

Test for tannins

Quinoa seed powder was treated with 15% ferric chloride test solution. The blue colour in the mixtures signified the presence of hydrolysable tannin. For confirmation, 0.5g of the extracts were added to 10 mL of freshly prepared potassium hydroxide (KOH) in a beaker and shaken to dis-

solve. A dirty precipitate was indicative of the presence of tannin.

Test for steroids (Lieberman-Buchard reaction)

About 200 mg of quinoa seed powder was once dissolved in two ml chloroform. Sulfuric acid used to be carefully added to form a lower layer. A reddish-brown colour at the interface used to be indicative of the presence of steroidal rings.

Test for flavonoids

Three methods were used to determine the presence of flavonoids in the quinoa seed powder. A total of 5 ml of 10% ammonia solution was added to a portion of the aqueous filtrate from the shoot extract of the cover crops followed by addition of concentrated H₂SO₄. A yellow colouration observed in each extract was used to validate the presence of flavonoids. About 200 mg of quinoa seed powder was once dissolved in two ml chloroform. Sulfuric acid used to be carefully added to form a lower layer. A reddish-brown colour at the interface used to be indicative of the presence of steroidal rings.

Test for cardiac glycosides (Keller-Killani test)

A total of 2 gm of quinoa seed powder was treated with 2 ml glacial acetic acid containing one drop of ferric chloride solution. This used to be underlaid with one ml of concentrated sulfuric acid. The formation of a brown ring of the interface was indicative of the presence of a deoxy sugar of cardenolides.

Test for terpenoids (Salkowski test)

A total of 5 gm of quinoa seed powder was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to separate the 2 layers distinctly. A reddish-brown colouration of the interface was formed to confirm the presence of terpenoids.

Test for anthraquinone (Borntrger's test)

About 0.5 g of the quinoa seed powder was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract used to be filtered and the filtrate was shaken with an equal quantity of 10% ammonia solution. A red-violet or purple colour in the ammonia layer was observed which indicates the presence of anthraquinones.

Determination of Vitamins and Minerals

Minerals (Magnesium, Zinc, Manganese, Vanadium and Chromium) and Vitamins (Vitamin -D and Vitamin -E) were analysed with an atomic absorption spectrophotometer (AAS; Shimadzu Instruments, Inc., SpectrAA-220).

Experimental Design

Adult male albino Wistar rats (6 weeks), weighing 150 g to 200 g were used for the present antidiabetic study. The animals were housed in clean polypropylene cages and maintained in a well-ventilated temperature-controlled animal residence with a regular 12 h light/dark schedule. The animals had been fed with preferred rat pelleted diet (Hindustan Lever Ltd., Mumbai, India) and clean ingesting water used to be made available ad libitum. All animal procedures were performed after approval from the ethical committee IAEC NO: KMCRET/Ph.D/10/2017-2018 and following the recommendations for the proper care and use of laboratory animals.

The animals were divided into four groups of six animals each. The rat 6 weeks animals are kept overnight fasting and Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Non-insulin subordinate diabetes mellitus was incited in for the time being abstained rodents by a solitary intraperitoneal infusion of 60 mg/kg streptozotocin, 15 min after the i.p organization of 120 mg/kg of nicotinamide.

Hyperglycemia was confirmed by the elevated levels of blood glucose were determined at 72 hrs. The animals with blood glucose level more than 250mg/dl will be used for the study.¹⁴

The Six experimental groups with six rats each prepared as per given schedule.

Group 1: Control only normal saline

Group 2: Only Streptozotocine 60 mg/kg/b.w. (IP) +Nicotinamide 120mg/kg (po)

Group 3: Streptozotocin (60 mg/kg) Nicotinamide 120mg/kg (po) rats treated with

Glibenclamide 20 mg/kg(po)

Group 4: Streptozotocin (60 mg/kg) +Nicotinamide 120mg/kg (po) rats treated with

Chenopodium quinoa 150 mg/Kg.

Group 5: Streptozotocin (60 mg/kg) +Nicotinamide 120mg/kg (po) rats treated with

Chenopodium quinoa 250 mg/Kg.

Group 6: Streptozotocin (60 mg/kg) +Nicotinamide 120mg/kg (po) rats treated with

Chenopodium quinoa 350 mg/Kg

The fasting animal body, blood glucose level used to be estimated on 1st,7th,14th, 21st and 28th day. At the end of experimental period rats were fasted overnight and anaesthetized with Ketamine; blood samples were collected through retro-orbital sinus puncture after 90 min administration of

treatment doses to the respective group animals with or without EDTA container for biochemical parameters estimation. Fasting blood glucose level used to be measured by using Accu-Check active blood glucose meter (Roche Group, Indianapolis, In USA)

Histopathologic Analysis

The pancreases were fixed for 48 hrs in 10% formalin saline and processed through the paraffin technique. Sections of 5-micron thickness have been cut and stained by using hematoxylin and eosin (H & E) for histological examination. Observation of slides was once performed under a light microscope (DM2000; Leica, Bensheim, Germany).

Statistical Analysis

The data were analysed by one-way ANOVA followed by Dunnett test. $p < 0.05$ was considered as a level of significance. The resulting values were expressed as mean \pm standard error of the mean.

RESULTS

The phytochemical screening of hot and cold-water extracts of quinoa seed powder revealed the presence of alkaloids, flavonoids, saponins, proteins, carbohydrates and showed the absence of tannins, cardiac glycosides, steroids. (Table 1). The powdered quinoa seeds were tested for Minerals (Magnesium, Zinc, Manganese, Vanadium and Chromium) and Vitamins (Vitamin -D and Vitamin -E) were measured with an atomic absorption spectrophotometer (AAS) (Table 2).

Table 1: Phytochemical analysis of Quinoa in Aqueous solution

S. No	Phytochemical Constituents	Specific Test	Hot Water	Cold Water
1	Alkaloids	Wagner's Test	+	+
2	Flavonoids	Alkaline Reagent Test	+	+
3	Saponins	Foam Test	+	+
4	Tannins	Ferric Chloride Test	-	-
5	Cardiac glycosides	Keller - Killiani Test	-	-
6	Steroids	-	-	-

+ " presence, " - " absence

A large number of minerals found in quinoa seeds. The present results in Table 2 showed that quinoa seeds powder had the content of Magnesium, Zinc, Chromium, Manganese, and Vanadium as follows 682.33 ppm, 20.97 ppm, 1.07 ppm,

9.69 ppm, 0.51 ppm respectively. These minerals are considered to make sufficient balance diet.

The results are given in Table (2) indicates that quinoa seeds contain a considerable amount of Vitamin E 47.0 ppm and Vitamin D shows the less than the limit of 10.0 ppm. Low levels of Vitamin E are related to an increased incidence of diabetes, and some research suggests that human beings with diabetes have lower levels of antioxidants.

Quinoa has been currently used as a source to maintain sugar levels. In the hypoglycemic activity, the end of the experiment (6 weeks), glucose was determined immediately after sacrificing. The glucose value was decreased significantly as shown in Table 3 in the diabetic rats that had (150 mg – 350 mg/Kg) quinoa powder in the administered diet compared to the diabetic rats treated with Glibenclamide Standard.

Table 2: The Minerals and Vitamin Contents of Quinoa Seed Powder.

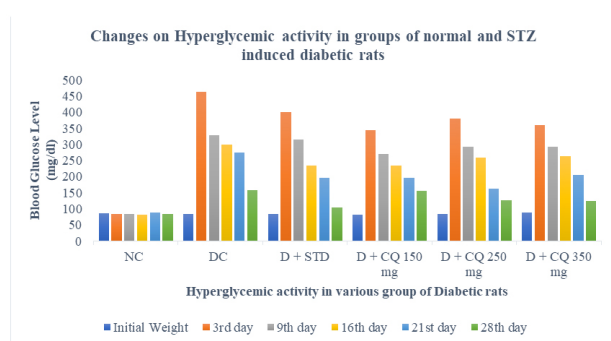
S. No	Parameters	Units	Values
1	MINERALS		
1.a	Magnesium	ppm	682.33
b	Zinc	ppm	20.97
c	Chromium	ppm	1.07
d	Manganese	ppm	9.69
e	Vanadium	ppm	0.51
2	VITAMINS		
2.a	Vitamin - D	ppm	<10.0
b	Vitamin - E	ppm	47

The untreated diabetic control group showed an increase in blood glucose level throughout the entire study period. Initially, the blood glucose level of the diabetic control group was 463.33 ± 96.80 and after 28 days of the trial period, the blood glucose level was decreased to 158.33 ± 51.85 . Figure 1 shows the effect of different concentration of quinoa seed powder up to 28 days at the dose of 150, 250 and 350 mg/kg significantly enhance the alterations in blood glucose levels in streptozotocin prompted diabetic rats. The concentration of 250 mg/kg of Quinoa seed powder showed significant antidiabetic activity 380 ± 86.87 was decreased to 126.66 ± 28.12 mg/dl against streptozotocin-induced diabetic rats and the effect was comparable with that of the standard drug glibenclamide (60 μ g/kg). After the treatment with the combination of quinoa seed powder and glibenclamide, there was a significant decrease in blood glucose level.

Table 3: Hyperglycemic activity of Quinoa seeds in STZ induced diabetic rats.

Groups	Pre-treatment			Post-treatment		
	Initial Glucose	3 rd day	9 th day	16 th day	21 st day	28 th day
NC	85.83± 2.3	83 ± 3.11	83 ± 2.30	81.16±2.53	87.33±1.60	84.66±2.40
DC	85 ± 7.07	463.3± 96.8	328.33±66.2	300± 96.36	275± 87.58	158.33±51.85
D + STD	84.16± 3.0	400±46.54	315.83±22.9	235± 51.55	195±41.12	105 ± 23.48
D + CQ 150 mg	80.83±3.9	343.33±79.7	270 ± 58.42	23 ± 75.97	196.66±64.16	156.66±51.48
D + CQ 250 mg	85 ± 3.16	380 ± 86.87	293.33±62.6	260± 54.83	161.66±52.56	126.66±28.12
D + CQ 350 mg	88.33±5.27	360 ± 78.86	291.66±61.5	263.3± 53.5	205 ± 43.64	123.33±39.55

NC – Normal Control, DC – Diabetic Control, D + STD – Diabetic treated with Glibenclamide, D + CQ 150mg – Diabetic treated with *Chenopodium quinoa* at 150mg/kg, D + CQ 250 mg - Diabetic treated with *Chenopodium quinoa* at 250mg/kg, D + CQ 350mg - Diabetic treated with *Chenopodium quinoa* at 350mg/kg.

**Figure 1:** Hyperglycemic activity in Initial glucose level and 28th day

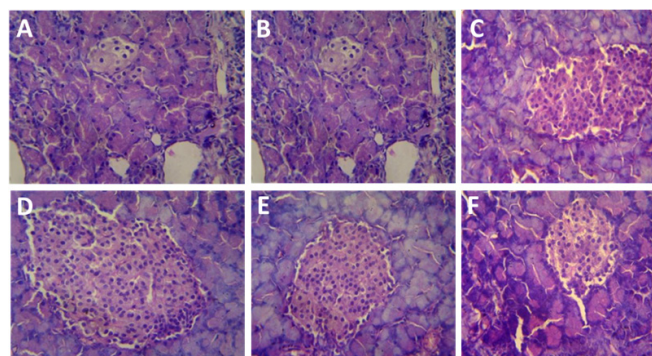
Histopathological examination results

The pancreas of normal rats showed no histopathological changes (Figure 2A). Besides, the pancreas of diabetic rats showed interlobular inflammatory cells infiltration (Figure 2B). The pancreas of diabetic rats treated with Glibenclamide (STZ – STD) pancreas showing adequate protection from HFD-induced changes in the pancreatic islet's cells (Figure 2C). However, Pancreas of diabetic rats fed on 150 mg and 250 mg quinoa powder (Figure 2D and Figure 2E) showed normal pancreatic acini. Islets are normal in number with focal cytoplasmic vacuolation. Finally, the pancreas of diabetic rats fed on 350 mg shows normal acini. Islets are decreased in size and number with cytoplasmic vacuolation and scattered inflammatory infiltrates (Figure 2F).

DISCUSSION

Additionally, magnesium assists with relaxing up veins and consequently to reduce headaches. It might likewise lessen Type 2 diabetes by advancing sound glucose control alongside giving other medical advantages. Zinc is a cofactor in numerous compounds that alter development and advancement, sperm age, processing and nucleic corrosive amalgamation. Chromium is required for the preservation of regu-

lar glucose metabolism. Experimental chromium deficiency leads to impaired glucose tolerance, which improves upon the addition of chromium to the diet. In humans' pharmacological doses alter lipid and glucose metabolism by way of improving glucose oxidation, glycogen synthesis and hepatic glucose output. Vanadium acts primarily as an insulin-mimetic agent, although enhanced insulin activity and improved insulin sensitivity have also been noted. More current research suggests that insulin may additionally be required for its effects.

**Figure 2:** Pancreas of rat from **A)** normal rats showing no histopathological changes. **B)** diabetic rats showing interlobular inflammatory cells infiltration, **C)** diabetic rats treated with metformin showing vacuolation of cells of islets of Langerhan's, **D)** diabetic rats fed on 150 mg quinoa powder showing no histopathological changes, **E)** diabetic rats fed on 250 mg quinoa powder showing no histopathological changes, **F)** diabetic rats fed on 350 mg quinoa powder showing cytoplasmic vacuolation and scattered inflammatory infiltrates.

Quinoa is a decent wellspring of calcium, iron, potassium, magnesium and zinc when contrasted with everyday mineral proposals. It tends to be said that on normal quinoa is a preferred wellspring of minerals over most grains. Nutrients are intensifying fundamental for the soundness of people groups and creatures. Customarily, nutrients have been among the

most broadly applied synthetic specialists to improve the dietary benefits of food products.¹⁵ Quinoa furthermore conveys a critical amount of nutrient E, which go about as a cell reinforcement, even though the degree decreases after handling and cooking.

Our information is in line with Graf et al., who referenced that when fat, hyperglycemic mice were given an enhancement made by draining supplements from quinoa seeds, their fasting glucose dropped.¹⁶ Similarly, a quinoa-fortified food regimen decreased blood sugar levels compared to those without quinoa supplementation.

Controlling eating regimens is for the most part considered as controlling of diabetes, and quinoa is one of these weight controls plans as an extraordinary decision for controlling diabetes. It is one of the different parts of a sound diabetic eating routine just as foods grown from the ground, lean proteins and unsaturated fats. Quinoa does have fructose and glucose ranges comparatively minimize than starches in different grains. The integral fatty acids in quinoa are the kinds that have been linked to enhancing insulin sensitivity (i.e. insulin is better able to get glucose out of the blood circulation and into cells.¹⁷

As cited in previous studies, quinoa as a fibre-rich-source plays an important role that influences blood sugar besides keeping body weight to prevent chronic illness accompanied by diabetes, and fibre has been proven to enhance the response of insulin after consuming, announced that taking care of quinoa in an eating routine to rats on a high-fructose diet, diminished the greater part of the unfavourable impacts brought about by fructose, which are all related with type 2 diabetes.¹⁸⁻²⁰ Valencia-Chamorro reported 2% mono-saccharides and 2.3% disaccharides in quinoa. Maltose and d-xylose are found in quinoa flour with high percentages while it has a lower concentration of glucose and fructose which allows its use in the malted drink.²¹

CONCLUSION

Incorporation of quinoa in our diet not only increases the nutritive value alternatively will also minimize the risk of various health illness like cardiovascular diseases, type 2 diabetes, excessive blood pressure, cancer, obesity. Quinoa is additionally a gluten-free elective food accessible for celiac patients. Being exceptionally nutritious, quinoa can be utilized to supplement the eating routine of that populace who are battling from lack of healthy sustenance, are adversely affected by certain nutritional categories or are just vegetarians. Utilization of quinoa speaks to a promising territory of examination as its utilization in our day by day diet can improve the admission of certain significant supplements and phytochemicals which caters significant medical advantages.

The blood glucose esteem was reduced essentially in the diabetic rodents that had (150 mg – 350 mg) quinoa powder treated contrasted with the diabetic control rodents that they show it an enormous advantage over different yields as far as human sustenance and wellbeing support. Quinoa seeds ought to be suggested for usage on a business scale in the Saudis dinners and processing plants since such seeds can give more assurance against diabetic illness. At long last, it very well may be presumed that by expanding the mindfulness concerning quinoa's biodiversity, ability to support in various development techniques, its different culinary uses and most significant its ability to cook tremendous medical advantages, the upgrade in the ailment of the huge fragment of the helpless populace of this world can be improved.

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