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Quinoa seed: A source of lipophilic nutraceuticals for the prevention of metabolic syndrome in a rat model

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SUMMARY: Metabolic syndrome (MS) is a cluster of metabolic changes including hypertriglyceridemia, elevated glucose tolerance and fatty liver. The aim of this research was to study the bioactivity of petroleum ether extracts prepared from quinoa 1 and Hualhuas quinoa in a MS rat model. Fatty acids and α-tocopherol were assessed in the extracts. MS was induced by feeding a high fructose-high fat diet (HFFD). Four groups of rats were assigned: the control group, fed a balanced diet; the control group, fed a HFFD diet; and two test groups, fed on a HFFD diet and treated by either quinoa 1 or hualhuas extract. The Glucose tolerance, plasma lipids, oxidative stress biomarkers, liver lipids and histopathology of the liver and heart were assessed. The results showed that extracts from both quinoa varieties had the potential to prevent MS; although quinoa 1 was more effective. In both varieties, the major fatty acid was linoleic. Hualhuas showed a higher percentage of linolenic acid than quinoa 1; while more alpha-tocopherol was present in quinoa1.

***KEYWORDS*:** *Fatty acids; Fructose; Lipophilic extracts; Metabolic syndrome; Quinoa seed; Tocopherol.*

RESUMEN: ***Semilla de quinoa: fuente de nutracéuticos lipófilos para la prevención del síndrome metabólico en modelo de rata.*** El síndrome metabólico (SM) es un conjunto de cambios metabólicos que incluyen hipertrigliceridemia, tolerancia elevada a la glucosa e hígado graso. El objetivo de la investigación fue estudiar la bioactividad de extractos de éter de petróleo preparados a partir de quinoa 1 y quinoa Hualhuas en modelo de rata con SM. En los extractos se evaluaron los ácidos grasos y el α-tocoferol. El SM se indujo mediante la alimentación con una dieta alta en fructosa y grasas (HFFD). Se asignaron cuatro grupos de ratas. El control se alimentó con una dieta equilibrada, otro grupo se alimentó con una dieta HFFD y dos grupos de prueba alimentados con HFFD se trataron con quinoa 1 o extracto de hualhuas. Se evalua**ó** la tolerancia a la glucosa, los lípidos plasmáticos, los biomarcadores de estrés oxidativo, los lípidos hepáticos y la histopatología del hígado y el corazón. Los resultados mostraron que los extractos de ambas variedades de quinoa tenían el potencial de prevenir el SM; aunque la quinoa 1 fue más efectiva. En ambas variedades el ácido graso principal fue el linoleico. Las hualhuas mostraron mayor porcentaje de ácido linolénico que la quinoa 1, mientras que la quinoa 1 presentó más alfa-tocoferol.

PALABRAS CLAVE: *Ácidos grasos; Extractos lipófilos; Fructosa; Semilla de quinoa; Síndrome metabólico; Tocoferol.*

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1. INTRODUCTION

Metabolic syndrome (MS) is of high prevalence worldwide and represents a public health problem with elevated costs. The syndrome has not been fully understood in terms of its multifaceted origin, although a sedentary life style and unbalanced dietary pattern might be the most common factors in its development (Lemieux and Després, 2020; Bovolini *et al*., 2021). MS is an aggregate of clinical conditions including abdominal obesity, hypertriglyceridemia, high density lipoprotein-cholesterol, high blood glucose, hypertension, and elevated inflammatory cytokines and is often associated with fatty liver. Therefore, MS is linked to an increased risk of chronic diseases, especially cardiovascular diseases (CVDs) and type 2 diabetes (Lemieux and Després 2020; Bovolini and García, 2021). Current therapy for MS is limited to the individual management of hypertriglyceridemia, hypertension, and hyperglycemia, in addition to controlling diet with regular exercise. The beneficial effects of nutraceuticals in MS are worth investigating. Nutraceuticals are bioactive ingredients from foods that possess prevention or treatment of one or more chronic diseases. Quinoa seeds might be a good source of nutraceuticals which may have health benefits towards MS. Quinoa is called a pseudo-cereal from a botanical point of view, due to its exceptional balance between protein, oil, fibers and carbohydrates, in addition to the presence of phytochemicals and variable bioactive constituents. Therefore, quinoa is considered a functional food that may lower the risk of chronic diseases (Vega-Gálvez *et al.,* 2010). It is an important source of minerals, vitamins, polyphenols, phytosterols, flavonoids, vitamin E and omega-6 fatty acids with possible nutraceutical benefits (James, 2009). In a previous work, quinoa alcoholic extracts (Hydrophilic extract) were shown to prevent steatohepatitis and CVDs (Al-Okbi *et al*., 2021). Therefore, in the present study, the health benefits of the other compartment of quinoa, the lipophilic extract, were investigated. Due to the reported presence of the aforementioned anti-inflammatory, hypolipidemic, and antioxidant constituents in the lipophilic compartment of quinoa, it was hypothesized that the petroleum ether extract of quinoa might have potential beneficial effect towards MS. Hence, the objective of the present research was to study the lipotropic activity (promoting the removal of fat from the liver), the lowering effect on postprandial glucose, the antioxidant and the correction of dyslipidemia by petroleum ether extracts from two quinoa varieties in a rat model of MS. Investigations of the histopathological changes in the liver and heart in MS rats and the consequent effect of quinoa extracts were among the aims of the present study. This research also aimed at associating biological activity with the content and profile of α-tocopherol and fatty acids, respectively, in both quinoa varieties.

2. MATERIALS AND METHODS

2.1. Materials

Two varieties of wild *Chenopodium quinoa* seeds from the Amaranthaceae family were studied in the present work: quinoa 1, which was obtained from the Agriculture Research Center, Giza, Egypt; and Hualhuas, which was supplied from the International Potato Center (CIP), Lima, Peru.

2.2 Methods

2.2.1. Preparation of petroleum ether extracts from two Chenopodium quinoa varieties

The quinoa seeds of the two varieties were separately washed with water and dried in a hot air oven at 40 ºC. Known weights of both seed varieties were extracted with a continuous extraction apparatus (soxhlet) by petroleum ether (40-60). After complete extraction, the solvent was evaporated at a temperature not exceeding 40 °C and the obtained extracts were used in the present study.

2.2.2. Determination of α-tocopherol and fatty acid methyl esters in the petroleum ether extracts of the two quinoa varieties.

The samples were saponified according to Lee *et al.* (2012) for the determination of α-tocopherol. The samples (2 g) were added to 15 ml of ethanol containing 6% pyrogallol in a saponification vessel and mixed by vortex for 30 seconds. After sonication for 5 min, 5 ml of potassium hydroxide (30%) were added and the vessel was flushed with nitrogen gas for 1 min. The contents were heated at 70 °C for 50 min in a shaking water bath. After the samples had been cooled for 5 min in an ice bath, 20 ml of sodi-

um chloride (2%) were added, and mixed by vortex for 30 seconds. The mixture was extracted three times with 50 ml n-hexane: Ethyl acetate extraction solvent (80:20, v/v containing 0.1 g/L BHT). The extracts were collected, diluted to a final volume of 50 ml, filtered through a 0.2 mm filter and analyzed by HPLC. Agilent Technologies 1100 series liquid chromatography equipped with degasser, quaternary pump, auto sampler, diode-array and florescence detectors were used. The analytical column was Eclipse XDB-C18 (150 X 4.6 µm; 5 µm). Alpha-tocopherol was identified and quantified by comparison with a known standard.

Methyl esters were prepared via trans-esterification of the fatty acids using a methanol sulfuric acid and chloroform mixture according to Indarti *et al*. (2005). Twenty mg of the oil were weighed into clean, 10-ml screw-cap glass tubes, to which 4 ml fresh solution of a mixture of freshly prepared methanol, concentrated sulfuric acid and chloroform (1.7/0.3/2 v/v/v) was added. The tubes were covered and trans-esterification was run at 100 °C for 30 min. On completion of the reaction, the tubes were brought to room temperature. Then, 3 ml distilled water were added to the mixture and thoroughly mixed by vortex for 1 min. After the formation of two phases, the lower phase containing the fatty methyl esters was transferred to a clean glass tube and dried with anhydrous Na_2SO_4. Fatty acids were analyzed by GC after the addition of a known volume of chloroform. The assessment of the methyl ester was carried out by injecting 2µl into a Hewlett Packard HP-system 6890 gas chromatograph equipped with FID. A HP-5 capillary column (30 m x 0.32 mm i.d.; 0.25 µm film thickness) was used to separate the different methyl esters. The chromatographic analysis conditions were as follows: initial temperature 70 ºC, held for 1 min, then raised to 120 ºC at a rate of 40 ºC /min, held for 2 min, then the temperature was finally raised to 220 ºC at a rate of 4 ºC /min and then held for another 20 min. The injector and detector temperatures were 250 ºC and 280 ºC, respectively. Identification of the fatty acid methyl esters was carried out by direct comparison of the retention times of each of the separate compounds to standards of the fatty acid methyl esters analyzed under the same conditions. Quantification was based on peak area integration.

2.2.3. Animals

Adult male albino rats of body weight ranging from 70 to 80 g (age: 5-6 weeks) were used in the present study. The animals were purchased from the Animal House of National Research Centre, Cairo, Egypt. The rats were housed in stainless steel cages (2 rats/ cage); water and food were given *ad-libtium* with a 12-hour light/dark cycle. The animal experiment was carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt, and followed the recommendations of the Health Guide for Care and Use of Laboratory Animals.

2.2.4. Diets

Two diets were prepared -- a balanced diet and a high fructose-high fat diet (HFHFD). The balanced diet was composed of 10% protein supplemented from casein, 10% sunflower oil, 70.5% starch, 5% cellulose, 1% vitamin mixture and 3.5% salt mixture. The HFHFD was prepared from 10% protein supplemented from casein, 20% sheep tallow, 63.5% fructose, 2% starch, 1% vitamin mixture and 3.5% mineral mixture. The high fructose-high fat diet was prepared in accordance with Al-Okbi *et al*. (2021) with some modification for the induction of metabolic syndrome (MS).

2.2.5. Preparation of the extracts in suitable form for rat dosing

The extracts were emulsified separately in water using tween 80 at 10% from the extract weight and the whole mixtures were mixed using vortex to be ready for dosing the test groups through a gastric tube. Similar quantities of water and tween 80 were mixed by vortex (representing the vehicle) to be given to the control groups.

2.2.6. Design of the animal experiment

Four groups of rats were assigned, each of 8 rats: control-fed balanced diet (NC), control-fed HFHFD (MSC) and two test groups fed the HFHFD and treated by either quinoa 1 or Hualhuas petroleum ether extract at a daily oral dose (500 mg/kg) for a month. The rats in the NC and MSC groups were given a daily oral dose from the vehicle. Body weight and food intake were measured

once weekly during the experiment. An oral glucose tolerance test (OGTT) was carried out after 4 weeks. After an overnight fast, blood was taken from the tails of all the rats for the determination of fasting blood glucose (zero time) using a Glucometer. Quinoa 1 and Hualhuas extracts were given to the rats in groups 3 and 4, respectively, orally at 500 mg/kg rat body weight; while the two control groups were given the vehicle. All the rats, including the two control groups, were given 1 g glucose/kg rat body weight in solution form (Al-Okbi *et al*., 2018). Blood glucose was assessed after 1/2 hr, 1hr, 2 hrs and 4 hrs from glucose intake. The rats continued feeding on the same diets and doses for an extra week. At the end of the experiment, nutritional parameters represented by total food intake, body weight gain and feed efficiency ratio (body weight gain/total food intake) were calculated. Blood samples were taken by heart puncture from fasted anesthetized rats and collected in heparinized tubes. The tubes were centrifuged and the plasma was separated. Anesthesia was carried out by an intraperitoneal injection of the pentobarbital (50 mg/Kg rat body weight). Plasma malondialdehyde (MDA) as a determinant of lipid peroxidation and total antioxidant capacity (TAC) was determined as an indicator of oxidative stress and antioxidant status, respectively, as previously assayed (Satoh, 1978; Koracevic *et al.,* 2021). Plasma lipids represented by total cholesterol (TC), triglycerides (TGs), low density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined in accordance with previous methods (Watson, 1960; Megraw *et al*., 1979; Schriewer *et al*., 1984; Burstein *et al*., 1970). The ratio of TC/HDL-C was calculated as cardiovascular disease risk factor. Rats were dissected after euthanasia through cervical dislocation under anesthesia. Livers and hearts were instantaneously removed from the dissected rats; a part of the liver was analyzed for total liver lipids according to the procedure of Cequier-Sànchez *et al*. (2008). Another part of the liver was kept with the hearts in 10% formalin for histopathological examination (Bancroft and Layton, 2019).

2.2.7. Statistical analysis

The results were expressed as the mean±SE. Normality of the data was confirmed by the Kolmogorov-Smirnov test, thus the parametric test of one-way analysis of variance ANOVA, followed by the Tukey test using the SPSS statistical program were applied. In all cases $p \leq 0.05$ was used as the criterion of statistical significance.

3. RESULTS

3.1. Fatty acids profiles and α-tocopherol in the petroleum ether extracts of quinoa

The fatty acid profiles are compiled in Table 1. It could be noticed that linoleic acid (omega-6) was the major fatty acid in both quinoa1 and hualhuas (46.895 and 58.800%, respectively). The second predominant fatty acid was oleic, a monounsaturated fatty acid, which showed 28.808 and 16.210% in quinoa1 and hualhuas, respectively. Linolenic acid (omega-3) demonstrated a higher level in hualhuas (8.981) compared to quinoa1 (1.261). Total unsaturated fatty acids were 76.964 and 83.991 in quinoa1 and hualhuas, respectively; while total saturated fatty acids were 11.533 and 10.239. The ratio Omega-3: Omega-6 was shown to be 1:37 and 1:7 in quinoa1 and hualhuas oils, respectively.

Table 1 demonstrates the levels of alpha-tocopherol in the petroleum ether extracts of both quinoa varieties. It was observed that quinoa 1 contained a very high level of alpha-tocopherol (787.638 µg/g) compared to Hualhuas (81.490 µg/g)

Table 1. Fatty acids as percentages of total fatty acids and alpha-tocopherol (µg/g) in both petroleum ether extracts of quinoa varieties.

	Quinoa variety	
	Quinoa 1	Hualhuas
Fatty acids		
C14:0 (Myristic)	0.426	-
C16:0 (Palmitic)	10.570	10.239
C18:0 (Stearic)	0.537	-
C18:1 (Oleic)	28.808	16.210
C18:2 (Linoleic; omega-6)	46.895	58.800
C18:3 (Linolenic; omega-3)	1.261	8.981
Total unsaturated fatty acids	76.964	83.991
Total saturated fatty acids	11.533	10.239
Omega -3: Omega- 6	1: 37	1: 7
Alpha-tocopherol (µg/g)	787.638	81.490

3.2. Results of the biological experiments

Glucose tolerance curves are shown in Figure 1. Insignificant changes in fasting blood glucose were noticed when all groups were compared to the NC group. The blood glucose levels of the MSC group of the different times intervals demonstrated significantly higher levels compared to the CN group. Rats treated with quinoa extracts exhibited a significant decrease in blood glucose throughout the determined intervals when compared to the MSC group, except for the group given the hualhuas extract after 4 h from glucose administration where the reduction was insignificant. All the levels of blood glucose in the rats given quinoa extracts during the glucose tolerance curve demonstrated insignificant changes from those of the NC group, except the group given hualhuas on 2- and 4- hour intervals, where they exhibited a significant increase.

The different plasma lipids, MDA, TAC and liver fat of the different groups are compiled in Figure 2. Significant increases in TG, TC, LDL-C and TC/HDL-C with significant reduction in HDL-C were manifested in the MSC group compared to the NC group. All lipid parameters showed significant improvements after treatment with quinoa extracts in comparison to the MSC group, except for only TG, in the case of the group being given the quinoa 1 extract. The rats treated with quinoa 1 extract showed a significant reduction in TC and LDL-C compared to those treated with hualhuas. The plasma MDA of the MSC group showed a significant elevation compared to the NC group. Both test groups demonstrated significant reductions in MDA compared to the MSC group. The plasma MDA of the group treated with quinoa1 exhibited a significant increase in comparison to the NC group; while hualhuas showed insignificant changes. The MSC group showed a significant reduction in TAC compared to the NC group. The groups treated with quinoa extracts demonstrated an insignificant increase compared to the MS control and a significant reduction compared to the NC group. Total liver fat of the MSC group demonstrated a significant increase compared to the NC group. The group treated with the quinoa 1 extract showed a significant reduction in total liver fat compared to the MSC group while demonstrating an insignificant change from the NC group. The Hualhuas extract-treated group only showed an insignificant reduction in total liver fat compared to the MSC group, while demonstrating a significant elevation in comparison to the NC group.

Figure 3 exhibited the nutritional parameters of different groups. Final body weight, body weight gain, total food intake and food efficiency ratio in the MSC group demonstrated insignificant changes in comparison to the NC group. The petroleum ether extracts of quinoa produced significant decreases in all nutritional parameters compared to either the MSC or the NC group, except for total food intake in

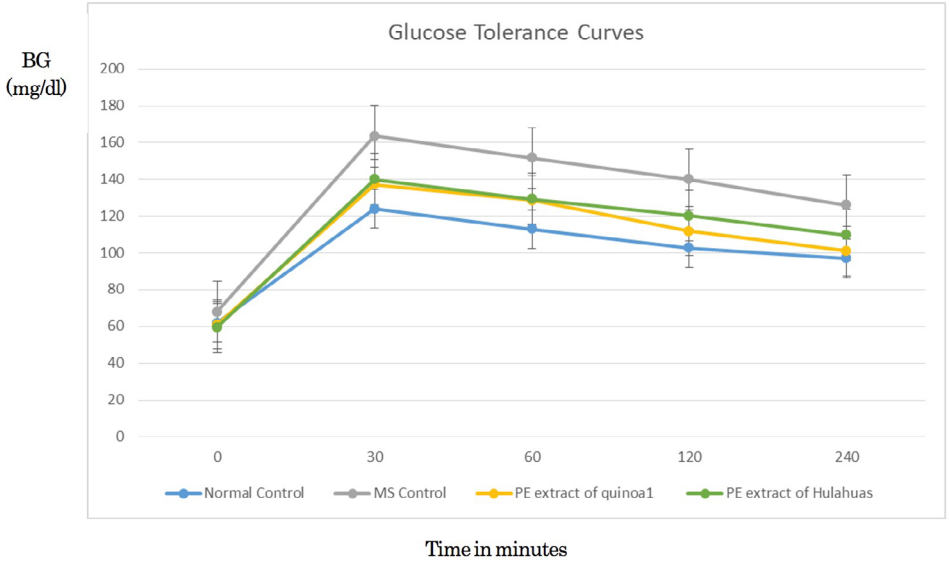

Figure 1. Fasting blood glucose and blood glucose after 1/2, 1, 2 and 4 hours from oral glucose administration as mg/dL of the different experimental groups. BG: Blood glucose, MS: Metabolic syndrome, PE: Petroleum ether. Each group of rats (n) = 8. Statistical ANOVA test was used.

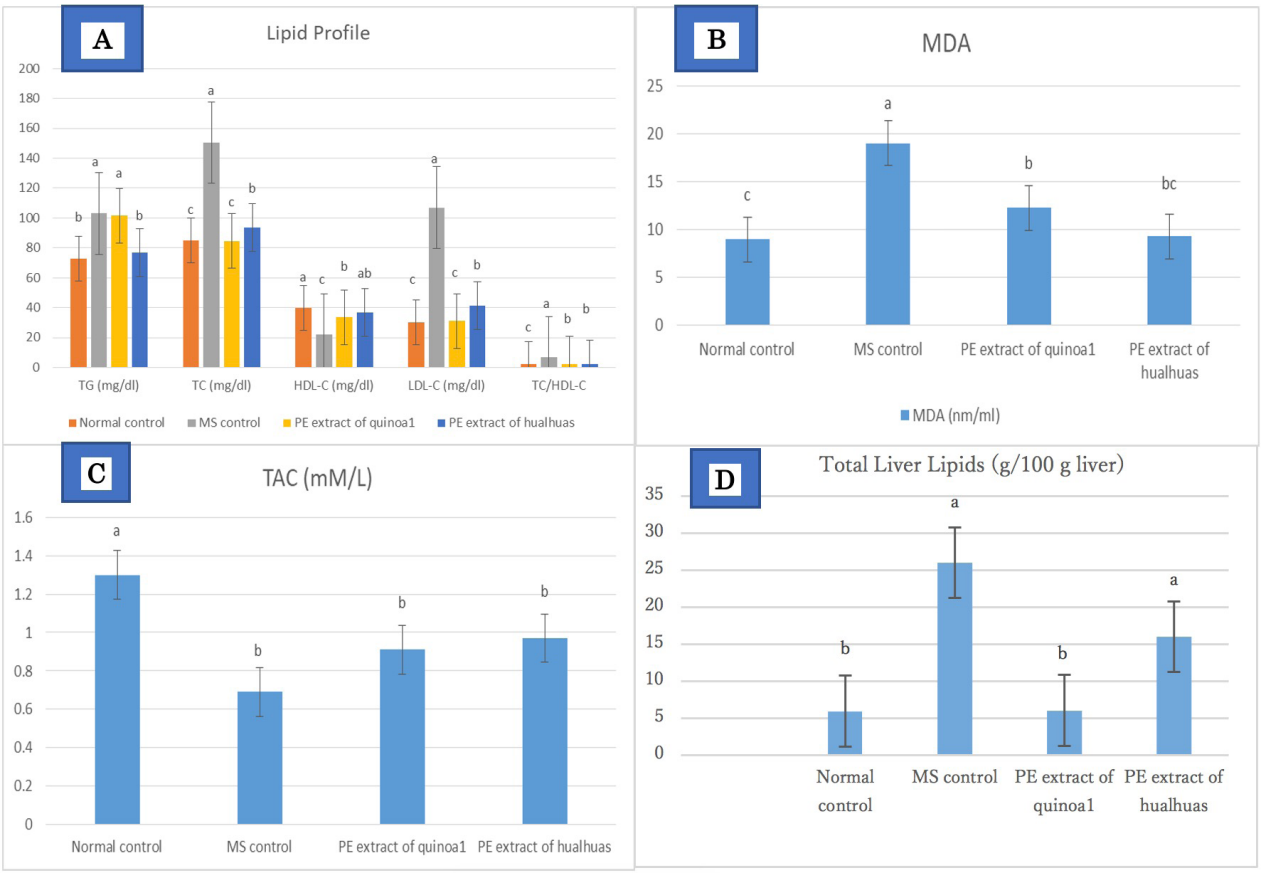

Figure 2. Plasma lipids (A), MDA (B) and TAC (C) and liver lipids (D) of different experimental groups. MS: Metabolic syndrome, PE: Petroleum ether, TG: Triglycerides, TC: Total cholesterol, HDL-C: High density lipoprotein-cholesterol, LDL-C: Low density lipoprotein-cholesterol, MDA: Malondialdehyde, TAC: Total antioxidant capacity. Each group of rats (n) = 8. Statistical ANOVA test was used: different letters mean significant difference, $p < 0.05$.

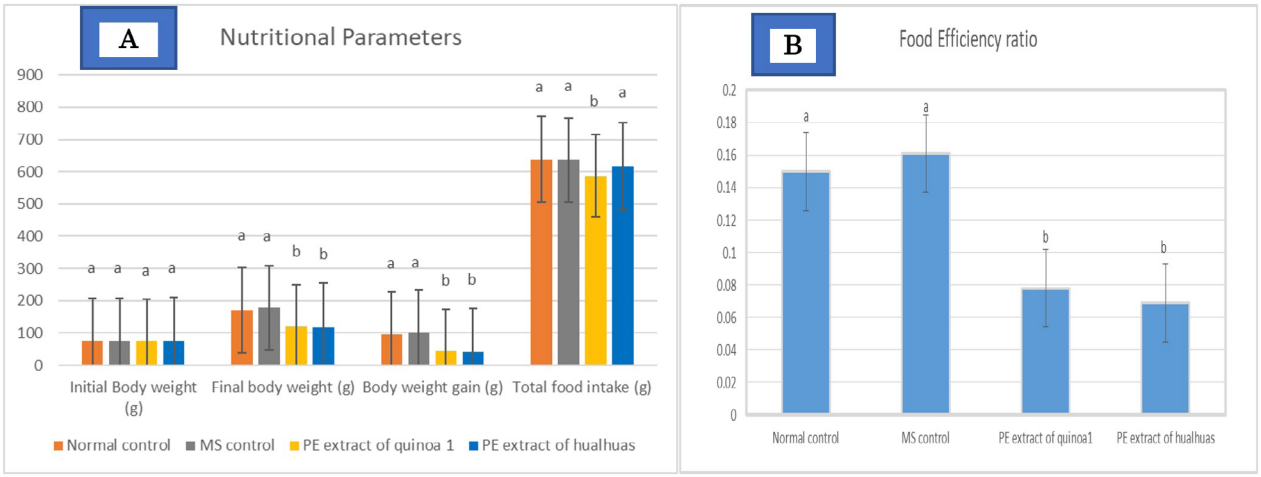

Figure 3. Nutritional parameters of rats from different experimental groups.
A: Initial body weight, final body weight, body weight gain and total food intake. B: Food efficiency ratio.
MS: Metabolic syndrome, PE: Petroleum ether. Each group of rats (n) = 8. Statistical ANOVA test was used: different letters mean significant difference, $p < 0.05$.

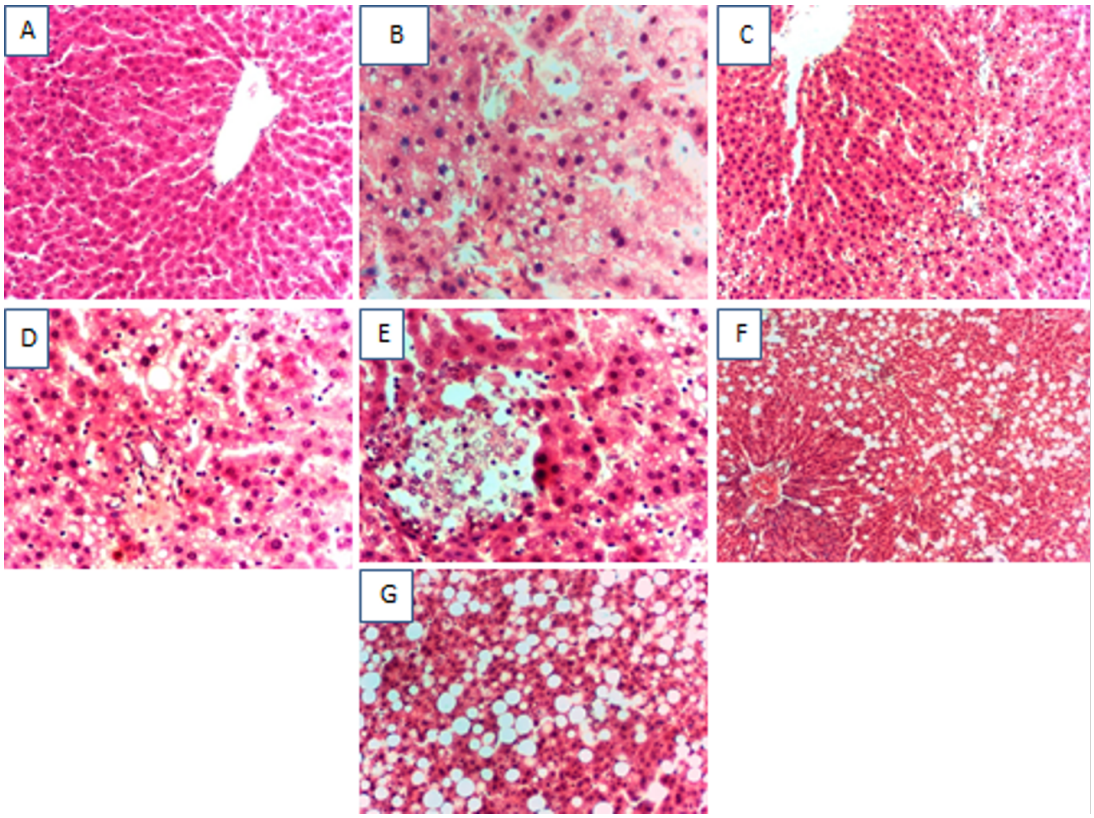

Figure 4. Histopathological changes in hepatic tissue of the experimental groups. A: Hepatic tissue of normal control group showed normal appearance of hepatic cells (H &E X200). **B:** Hepatic tissue of the metabolic syndrome control group showed great degeneration and vacuolation of hepatic cells, with deposition of fatty globules. The MSC group manifested slanted hepatic sinusoids together with focal necrosis. Inflammation was observed with infiltration of mononuclear inflammatory cells. Necrobiosis, pyknotic nucleus and deep homogenous eosinophilic cytoplasm, in addition to activation of kupffer phagocytic cells were also seen in the MSC group (H&E X200). **C:** Hepatic tissue of the group given quinoa 1 extract demonstrated from mild to moderate degeneration, vacoulation of the hepatic cytoplasm, with fatty changes in the portal area. Mild infiltration of mononuclear inflammatory cells was also seen (H&E. X200). **D:** High magnification of C where activation of kupffer cells was seen. (H&E. X400). **E**: Hepatic tissue of the group given quinoa1 extract showed cytoplasmic degeneration, vacoulation with fatty changes, Foci of necrosis with inflammatory cells (H&E. X400). **F:** Hepatic tissue of the group given hualhuas extract demonstrated marked and advanced degeneration, vacoulation of the hepatic cytoplasm, with fatty changes and large fat globules were diffused in hepatic tissue. The hepatic sinusoidal spaces were distorted (H&E. X100). **G**: Higher magnification of F showed diffused degeneration and vacoulation of the hepatic cytoplasm with fatty changes. (H&E. X200).

the case of the petroleum ether extract of hualhuas, which showed an insignificant change.

The histopathological changes in the liver of the different experimental groups are seen in Figure 4. It can be observed that the MSC group showed great degeneration and vacuolation of hepatic cells, with deposition of fatty globules. The MSC group manifested slanted hepatic sinusoids together with focal necrosis. Inflammation was observed with the infiltration of mononuclear inflammatory cells. The presence of necrobiosis, pyknotic nucleus and deep homogenous eosinophilic cytoplasm, in addition to kupffer phagocytic cell activation were also noticed in the MSC group. The group treated with quinoa 1 demonstrated mild to moderate histopathological changes compared to the MSC group; while rats given hualhuas did not produce any improvement in liver histopathology compared to the MSC group.

Figure 5 shows the histopathological changes in the heart in the different experimental groups. The NC group showed a normal appearance of the heart. The heart muscle of the control group with metabolic syndrome showed dispersed fragmentation together with muscle myofiber necrosis, nuclei pyknosis, and striation loss with interstitial edema. The longitudinal section of the myocardium of the group treated with the quinoa 1 extract exhibited minute focal necrosis, mild fragmentation and edema of the myofibers. The cross

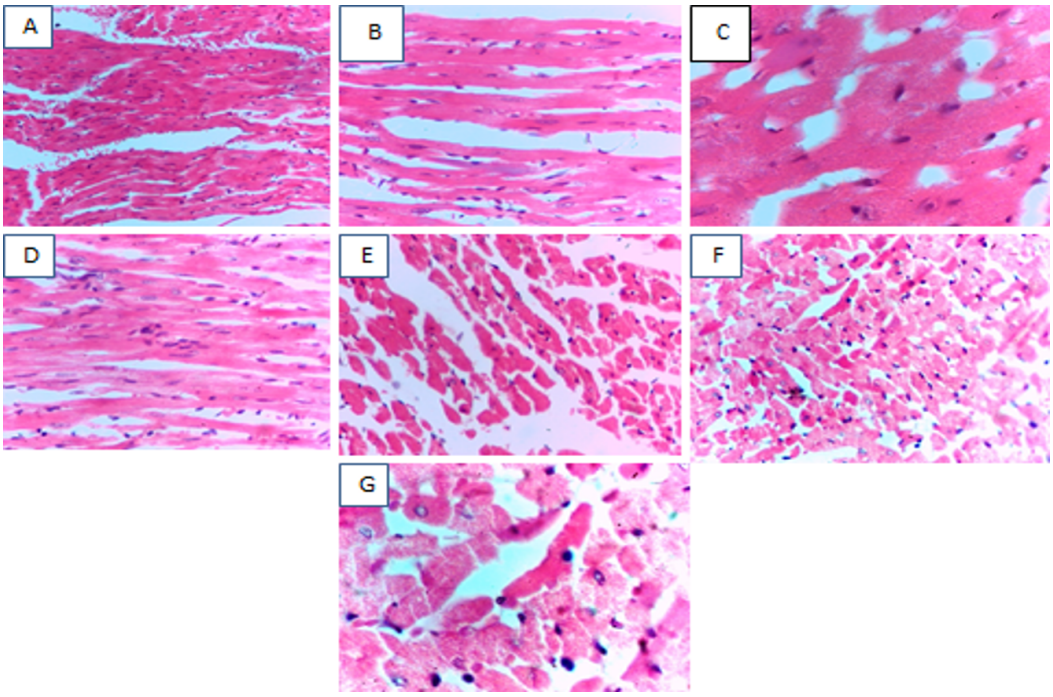

Figure 5. Histopathological changes in the heart tissue of the different groups.
A: Heart muscle of the control normal group showed normal appearance (H&E, X200). **B and C:** The heart muscle of the control group with metabolic syndrome showed dispersed fragmentation together with necrosis of muscle myofibers, pyknosis of the nuclei and loss of striation with interstitial edema (H&E, X 400 and X 1000, respectively). **D:** The longitudinal section of the myocardium of the group treated with quinoa 1 extract exhibited minute focal necrosis, mild fragmentation and edema of the myofibers (H&E, X400). **E&F**: The photomicrograph of the cross section of myocardium of the group given hualhuas quinoa extract presented necrobiosis of myofibers associated with pyknosis of the nuclei, loss of striation and fragmentation and interstitial edema (H&E, X200). **G**: Higher magnification of figure F (H&E, X1000).

section of the myocardium of the rats given hualhuas quinoa extract presented moderate myofiber necrosis accompanied by nuclei pyknosis, together with loss of striation, fragmentation and interstitial edema.

4. DISCUSSION

The demonstrated significant elevated glucose tolerance, dyslipidemia (high TG, TC, LDL-C and TC/HDL-C with low HDL-C), high MDA, low total antioxidant and elevated liver fat in addition to histopathological changes in the liver and heart in rats fed on HFHFD compared to the NC group indicated the induction of MS, which agreed with a previous study, (Al-Okbi *et al*., 2021). Elevated TGs, TC and LDL-C, along with a reduction in HDL-C were reported as risk factors for cardiovascular diseases (CVDs) especially on elevation of TC/HDL-C as could be seen on feeding HFHFD in the present study. Treatment with the extracts produced variable improvements in biochemical parameters and histopathology with a reduction in body weight compared to the MSC group. The therapeutic efficiency of both quinoa extracts resides in significantly reducing plasma TG, TC and LDL-C with the elevation of HDL-C. The reduction of TC/HDL-C on administration of the extracts pointed to the inhibition of cardiovascular disease risks. Liver fat was significantly reduced due to treatment with quinoa 1, which indicated the potential reduction of fat synthesis, elevated fat breakdown and /or lipotropic effect of such extract. The reduction of glucose tolerance and body weight might indicate the potential anti-diabetic and anti-obesity effects of the extracts, respectively. The reduction in body weight gain without affecting food intake in the case of treatment with the hualhuas extract might propose the presence of bioactive constituent with increasing energy expenditure. However, the reduction in food intake on administration of the quinoa 1 extract might point to the anorexigenic effect of such extract. The reduction of MDA on administration of the extracts revealed inhibition of lipid peroxidation with subsequent reduction of oxidative stress. The anti-MS effect of the extracts might be as-

cribed to the presence of alpha-tocopherol, omega-3 and omega-6 fatty acids as determined in the present study and other bioactive constituents reported previously. Gamma and α-tocopherol were identified in quinoa (Pereira *et al*., 2019; Tang *et al.,* 2016). In addition, phytosterols and carotenoids reported in previous studies in quinoa (Tang *et al*., 2016; Vega-Gálvez *et al*., 2010) might participate in such health benefits. The total phytosterols were reported to range from 9.4 to 12.2 g/kg (Shen *et al*., 2022). Trace amounts of α- and β-tocotrienols were found in quinoa (Tang *et al.,* 2015). Carotenoids, mainly trans-lutein and zeaxanthin, were identified for the first time in quinoa seeds (Tang *et al*., 2015). The antioxidant activities of lipophilic extracts were reported to be positively correlated with total carotenoids and total tocopherols (Tang *et al.,* 2015). Hypercholesterolemia was reported to produce a reduction in antioxidant enzymes (glutathione and catalase) causing damage to the oxidative defense system of the cell. Such changes lead to reactive oxygen species that lead to high oxidative stress (Nwichi *et al.,* 2012). Therefore, the hypocholesterolemic effect seen in the present study together with the reported antioxidant effect of quinoa could provide potential protection from CVDs.

It was reported that bread made from quinoa when consumed on a daily basis for a short term could modify glucose tolerance with a minimal effect on CVD risk biomarkers (Li *et al.,* 2018). In support of the previous studies, quinoa was shown to have potential in improving glucose tolerance (Mithila and Khanum, 2015 and Gabrial *et al*., 2016). Previous studies (Mithila and Khanum, 2015) demonstrated a reduction in body weight gain and food intake in rats fed quinoa, which agreed with the present study.

The oil content in quinoa ranges from 4.6 to 7.2%, (AAFRD, 2005; Koziol, 1993). The present work showed that the oil content in quinoa was 4.48 and 5.20% for quinoa 1 and hualhuas, respectively, which is similar to the abovementioned values. The two known essential fatty acids to humans are α-linolenic [Omega-3 (ω-3)] and linoleic [Omega-6 (ω-6)]. The essential fatty acids are metabolized in the body to long-chain fatty acids of 20-22 carbon atoms. Linolenic acid is metabolized to eicosapentaenoic (EPA) and docosahexanoic acid (DHA), while linoleic acid is metabolized to arachidonic acid. EPA and DHA are known for their anti-inflammatory and hypolipidemic effects (Al-Okbi *et al.,* 2020). Linoleic acid is the most predominant fatty acid in quinoa as can be seen from the present study. In a previous study, palmitic acid, oleic acid, linoleic acid and linolenic were shown to be present in quinoa at 10.66, 24.7, 62.47 and 2.19%, respectively (Altuna *et al*., 2018). In another study, Peruvian quinoa was shown to contain 50.2% linoleic acid, 26% oleic acid and 4.8% linolenic acid (Repo-Carrasco *et al.,* 2003). The present study showed that both varieties are rich in linoleic acid but only hualhuas contains appreciable amounts of linolenic acid (8.981%), while the variety quinoa 1 only contained a very low percentage (1.261). The fatty acid profiles of quinoa varieties in the current study somehow fall within the ranges of fatty acids reported by Altuna *et al*. (2018) and Repo-Carrasco *et al*. (2003) with only a higher value for linolenic in the hualhuas variety. The unsaturated fatty acids in quinoa in the present study are extremely high, nearly 7-8 times the saturated fatty acids. The ratio of omega-3/omega-6 fatty acids in quinoa was 1/6 (Shen *et al*., 2022), which somehow is equal to that determined in the present study concerning hualhuas (1/7), which more or less reflects a good ratio of health benefits as anti-inflammatory.

Polyunsaturated fatty acids, represented by ω-3 and ω-6, have several positive effects on cardiovascular disease, improved insulin sensitivity and as anti-inflammatory, although a balance in the ratio ω-3/ω-6 must be achieved (Bibus and Lands, 2015). All unsaturated fatty acids present in quinoa could be protected by the presence of vitamin E, identified in the present study, and which acts as a natural antioxidant which makes them more stable and less likely to become rancid, guaranteeing a longer shelf-life (Koziol, 1993). The content of vitamin E in quinoa is important for the human body since this vitamin acts as an antioxidant at the cell membrane level, protecting the fatty acids of the cell membranes against damage caused by free radicals. In a previous report, α-tocopherol was demonstrated to range from 2.6 to 5.37 mg/100 g quinoa (Vega-Galvez *et al*., 2010). These levels are very low compared to the α-tocopherol in quinoa varieties in the present study. The total tocopherol content in three varieties of quinoa ranged from 117.29 to 156.67 mg/kg and mainly consisted of γ-tocopherol (Shen *et al.,* 2022)

5. CONCLUSIONS

Petroleum ether extracts from both quinoa varieties showed the potential to prevent MS in rats via

improving dyslipidemia, glucose tolerance, cardiovascular risks and oxidative stress along with reducing body weight. Both extracts produced significant reduction in body weight gain, MDA and TC/HDL-C. Rats treated by either quinoa extract exhibited a significant decrease in blood glucose throughout the determined intervals when compared to the MSC group, except for the group given the hualhuas extract after 4 h from glucose administration, where the reduction was insignificant. Quinoa 1 extract possesses lipotropic effects through reducing liver fat. Also, the quinoa 1 extract was superior in reducing plasma TC, LDL-C and histopathological changes in the liver and heart; while hualhuas was more efficient in improving plasma TGs. The bioactivity of quinoa extracts might be attributed to the presence of alpha-tocopherol and omega-3 and omega-6 fatty acids as determined in the present study. The strengths of the present study reside in highlighting the beneficial effects of the petroleum ether extracts of two quinoa varieties on MS and the associated changes in the liver and heart as well as the possible bioactive constituents in the extracts represented by fatty acids and vitamin E. However, the weaknesses of the study might be the lack of detailed investigations into the different bioactive phytochemicals in the extracts. Therefore, future research could be addressed for the identification, isolation or fractionation of other different bioactive components present in such extracts which might play a role in protection from MS. In a prospective study, the extracts should be clinically evaluated in humans.

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7. DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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