Beneficial role of quinoa and *Nigella sativa* seeds as antihyperuricemia in rats

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ABSTRACT

This research targeted to evaluate the effect of Quinoa seed (QS); Nigella sativa seeds (NSS) and their combination on hyperuricemia. Thirty male albino rats assigned to five groups (n=6). The first these fed on the standard diet as normal control rats. The other rats (n=24), received a basal diet including a 20g /kg diet potassium oxonate to create hyperuricemia. Then ill rats divided into four groups. The hyperuricemic control group and three groups treated with added to their diet 10% QS; 4% NSS and mix from10% QS plus 4%NSS respectively. After that, the researcher calculated the biological status and estimated biochemical analysis. The results appear that high uric acid groups, in which feeding remedy diets showed no significant variation in biological parameters compared with the normal rats. The same groups indicated a significant improvement in renal function when compared with the injury group. Likewise, rising kidney tissues antioxidant status "superoxide dismutase; catalase and glutathione transferase" and decrease "malondialdehyde and interleukin-1 (IL-6)". Also, the mixture diet increased antioxidant activity. This study concluded that QS and NSS may improve kidney function and may reduce oxidative stress. This article recommended that special meals of hyperuricemic patients supplemented by a blend of QS and NSS.

Key words: quinoa seed, Nigella sativa seed, Hyperuricemia

INTRUDUCTION

Hyperuricemia is one of the evident spread of metabolic diseases in populations. It is famous for the rise of uric acid levels in the blood (**Chen et al., 2009**). This phenomenon may give an increase to problems, like gout (pilling up of uric acid crystals in the joints, especially in toes or fingers) and renal stones or kidney failure (**Kaneko et al., 2014**). Uric acid is the finished output of purine metabolism. Purines synthesized within the cells of the body. Also, it obtained from foods, which compounds containing nitrogen. Purines perhaps accumulate in tissues, forming crystals. These crystals lead to severe inflammation and cause tissue damage known through articular cartilage ulceration osteophytes and erosive lesions (**Corrado et al., 2006**).

Some cereals play a primary role in new lifestyles; to face consumer requests due to their physiological and metabolic advantage. Grain consuming has outspread from the breakfast list to any time of the day. These products have become an excellent way to insert functional food into the diet of consumers. Quinoa seed (QS) contains high biologically valuable "proteins, low glycemic index carbohydrates, phytoestrogens, and omega-3 and 6 fatty acids" that are beneficial to human health (**Farinazzi-Machado et al., 2012 and Ruini et al., 2015**). It has notable nutritional distinctive characteristics; not only from its protein content (15%) but also of its significant balance in amino acids (**Abugoch, 2009**) with the highest percentage of lysine (5.1% -6.4%) and methionine (0.4% - 1.0%) contents (**Jacobsen, 2003 and Jancurova et al., 2009**). Some researchers have suggested that QS may increase the secretion of immune substances called cytokines, which can be beneficial for both preventing and treating inflammation (**Yao et al., 2014**).

Nigella Sativaseed (NSS) is a very rich antioxidants source

ells influences many biological activities (**Rahmani and Aly 2015**). Thymoquinone is the main active ingredient of *NSS;* as an antioxidantitem and has anti-inflammatory properties (**Mahmoud et al., 2019**). *NSS* had numerous biological actions like many anti-cancer, anti-inflammatory, cardiovascular, renal, immunomodulatory, and anti-diabetic properties. Also, it acts as an antimicrobial, parasitic, and an antihypertensive. Besides its widespread use for the treatment of many diseases such as bronchitis, diarrhea, rheumatism and skin disorders (**Bamosa, 2015**).

The current research is a trial for evaluation of effective role of QSand *NSS*to mitigation hyperuricemia in experimental rats.

MATERIALS & METHODS

Materials:

The experimental seeds (QS and *NSS*) brought from the agricultural research center, Giza, Egypt. Quinoa seeds were washed for 20 minutes with tap water to eliminate toxic saponins and then dried at 45 ° C for 12 hours. These seeds were grinded in an electric blender into a fine powder, then packed in plastic bags and stored in the deep freezer until used for diet supplementation.

Chemicals:

Potassium oxonate gotten from El-Gomhorya Company, Cairo Egypt,Kits utilized for biochemical analyses fetched from Gamma Trade Company,Cairo Egypt.

Animals:

Thirty male albino rats of Sprague Dawley strain purchased from "Laboratory Animal Colonies, Pharmacology Department Faculty of Medicine, and Mansoura University"; the average weight was 120 ± 10g. Animals fed a standard/basal diet and water ready as needed. The rats left for 7 days to adapt according to (Guidelines for ethical conduct in the care and use of animals in research obtained from the

concerned department in Mansoura University).

Standard Diet:

Basal diet was prepared based on AIN 1993 **(Reeves et al. 1993)**.

Methods:

Chemical analysis:

The gross constituents of seeds as moisture, protein, fats and ash were estimated in accordance with (**A.O.A.C. 2005**) whereas carbohydrates were calculated by deduction. Minerals content including zinc (Zn), iron (Fe), sodium (Na), manganese (Mn), magnesium (Mg), cupper(Cu) and calcium(Ca) were determined according to (**Chapman & Pratt, 1978**)

The experimental design:

After acclimation on a basal diet for seven days rats classified into two groups. The first (n= 6) left as the normal control group, fed on standard diet. The second group (n= 24) rats were fed on basal diet contains 20g /kg diet potassium oxonate for 42 days to induce hyperuricemia as pointed by **Mazzali et al., (2001**). The uric acid level was measured for all rats at begin experiment and after six weeks of taking potassium oxonate to confirm the occurrence of hyperuricemia. A high uric acid was found in all rats that took potassium oxonate. Hyperuricemic rats classified into 4 groups as following: Hyperuricemic as a positive control group fed on a standard diet and water ad-libitium. Quinoa seeds group: received a basal diet containing 10% QS according to **(Ruales et al., 2002)** with an adjustment in the method by half the dose and double the duration. *Nigella sativa* group: intake on basal diet and 4% *NS*S. Mixed seeds group: fed on a basal diet containing 10% QS with 4% *NS*S according to **EL-Bahay et al., (2007).**

Biological items:

Daily feed intake (FI), protein intake (PI) and weekly body weight gain (BWG) werecalculated. Feed efficiency

ratio (FER) and protein efficiency ratio (PER) were specified according to the method of (**Chapman et al., 1959**).

At the ending of the trial term (35days), all rats were fastedovernight before sacrificing. Blood samples were taken from hepatic portal vein, one part was used for different determination and the rest blood was left in a centrifuge tube at room temperature for 15 min and then centrifuged at 4000 rpm for 10 min to obtain serum. Serum was separated in plastic vials and stored at -20C until analysis. Then, kidney were taken and homogenized in super cold 0.1M phosphate buffer at pH 8.0 (1: 4 w/v) using a homogenizer. Homogenate kidney was centrifuged at 4000 rpm for 15 min and supernatants were gathered to estimate antioxidants enzyme.

Biochemical analysis:

Determination of kidneys function "creatinine, urea and uric acid" were done according to **Bonsens and Taussky(1984), Patton and Crouch(1977) and Fossati et al., (1980).**Total protein, albumin and globulin were determination as described by method of **Weichse lbauml (1946), Bartholomev and Delany (1966) and Coles (1974)** respectively**.** An antioxidant enzyme in kidney tissue such as "Superoxide dismutase (SOD); glutathione peroxidase (GPXs); glutathione transferase (GST) and catalase (CAT)"estimatedas the method mentioned by **Beuchamp and Fridovich (1971); Tapple (1978); Moran et al., (1979) and Cohen et al.,(1970).** But, malondialdehyde determined according to **Uchiyama and Mihara, (1978**). Hemoglobin and packed cell volume were determined according to **Drabkin, (1949) and McInory (1954).** Whilst, immunoglobulin G and M (IgG and IgM) were determined by direct ELISA according to **Manohars and Selvakumaran, (2012).** Interleukin-6 (IL-6); IL-6 levels in kidney tissue homogenate was determined by using Rat IL-6 Immunoassay kit

from R&D Systems Inc. (USA) be of the same mind with **Hibi et al.,(1996).**

Statistical analysis:

Data were analyzed using analysis variance "ANOVA" test; (LSD) at $P \leq 0.05$ and Duncan's test as the methods described by **Gomez and Gomez, (1984).**

RESULTS & DISCUSSION

The chemical composition of QS and *NSS* explained in table "1". The major constituents of QS and *NSS* indicated that fat, protein and ash for NSS were higher than QS. Moisture and carbohydrates were the lowest in NSS. The results of the elementary chemical composition of QS were conformable with those stated by **Halaby et al., (2017)** whose demonstrated that moisture, fat, protein, ash and total carbohydrates were as follows: 9.5; 6.9; 14.2; 3.2 and 63.5 g/100g. Whiles, the results of the chemical components of the NSS were in line with **Atta, 2003** as follows; moisture, ash, oil, proteins, and total carbohydrates contents in the range of 3.8-7.0%, 3.7-4.7%, 22.0 to 40.35%, 20.85-31.2%, and 24.9-40.0%, respectively.

Data in Table (2) illustrated some minerals content of QS and NSS. The valueof minerals content *NSS* were displayed in table 2 lower than it's in QS. These results are congruent, with those acquired by **Atta, (2003); Nickavar et al., (2003); Ashraf et al., (2006) and Halaby et al., (2017**).

The findings in the third table indicated that the hyperuricemic rats recorded significantly lower results in BWG, FER, and PER than the healthy rats. PI and FI consequences have no significant differences in the positive control group, the negative control group, and all treated groups. The improvement of ill animals fed QS; NSS and mixture them showed significantly higher in BWG, FER, and PER value than a high uric acid group. These outcomes were maybe because

of the more content nutritional values of QS like zinc, magnesium, copper, iron, and calcium (showed in Table 2). Furthermore, potassium, magnesium, and calcium are present in it with bioavailable forms, and therefore their contents are sufficient for a balanced diet (**Vega-Galvez, et al., 2010**). QS is also a rich source of flavonoids that improves the biological functions of their antioxidant properties. These inferences are in harmony with those obtained by **Ruales et al. (2002)**. The improvement of biological sings in rats groups that consumed *NSS* was due to its nutrient content and thymoquinone substance **(Takruri and Dameh, 1998)**. This study revealed that the best biological parameters were in the group that consumed a blend of QS with *NSS.*

The datum in Table (4) explained that the hyperuricemic control group noted significantly high results in uric acid, urea, and creatinine in comparison to normal rats. While animals fed three remedy diets showed a significant reduction in renal function in differential to an injury control group. The best improvement was for the group that consumed a mixture of QS with NSS, followed by *NSS* only then QS. Rising uric acid value in serum could stimulus risk, like renal failure or kidney stones and gout (**Wang et al., 2015).** The data was in line with **Halaby et al., (2017)** they concluded that diet fortified QS can improving in the serum uric acid, urea, and creatinine levels. The results of improvement in kidney function may be attributed to thymoquinone that is the main active compound found in *NSS*. It has a probable capacity to preserve kidney action, rein the formation of urinary stones, lower retention crystals in the kidney tissue and spur their excretion in urine (**Benhelima et al., 2016)**.

QS or *NSS* contain some important compounds that help improve renal health as phenolic compounds, flavonoids, minerals, and vitamins. Dietary flavonoids diminish uric acid

levels and keep a safe kidney from damage, the mechanism underlying this effect is probably, due to their molecular structure, these natural antioxidants act as direct superoxide scavengers and xanthine oxidase (XO) inhibitors, resulting in the suppression of Reactive Oxygen Species (ROS) and uric acid formation **(Lin et al., 2015)**.

Table (5) recorded that hyperuricemic rats had significantly lower values of total protein (TP), albumin, and globulin than normal rats. Suffering hyperuricemic rats, which fed on a treated diet with QS or NSS and a mixture of both showed non-significant variation in TP, albumin, and globulin in comparison with normal rats. However, noted these groups a significant increase compared with the hyperuricemic control group. Low blood protein values in rats with elevated uric acid where uric acid is tightly merged to α1-α2 globulin, even though in little amounts (about 0.1-0.2 mg / dl). The Link of the other protein is loose, allowing glomerular uric acid to be filtered. Prior researches indicate that albumin is an assessment of uric acid value and is confirming in laboratory research suggesting that 1 g /dl of albumin shall combine with 0.6 mg/dl of uric acid (**Adams et al., 1984)**.

The results in table 6 illustrated that the hyperuricemic rats' had significantly lower SOD, CAT, GPXs, and GST value of kidney tissue while MDA was highly significant compared with normal rats. Hyperuricemia rat groups fed on diet Supported with QS or *NSS* and together it showed a significant increase of SOD, GPXs, GST, and CAT, however a significant reduction MDA in comparison with hyperuricemic control. One of the main defense systems that reduce the occurrence of toxicity caused by cracks in the body are antioxidants QS is a rich source of flavonoids that improve the biological functions of their antioxidant properties (**Abderrahim et al., 2015)**. The balance between antioxidants

and free radicals is a necessary and effective process in removing oxidative stress in intracellular organelles. The rats treated by QSplus *NSS* have shown an increase in the activity of these enzymes, demonstrating their effective ability to prevent the harmful effects of free radicals; thymoquinone is amongst the main antioxidant components of *NS*S.The intake of *NS*S in all forms develops the antioxidant defense capacity of the body (**Ermumcuand Sanlier2017**). The present results in table 6 has been confirmed by **Pasko et al., (2010)** who reported that QS can appear like evenly safeguard agents for rats by diminution the lipid peroxidation in plasma and different tissues and rising the antioxidant capacity. Also, the findings are in agreement with **Rahmathulla (2013)** who mentioned that the first line of cellular defense against oxidative harm is scavenger free radical enzymes like superoxide dismutase, glutathione peroxidase, glutathione transferase, and catalase. Reduced enzymes may be due to enhanced lipid peroxide.

Table 7 data indicated that a significant reduction for the HB, PCV, IgM, and IgG values; while the interleukin-1 (IL-6) level was highly significant of the hyperuricemic rats. These signs were due to potassium oxonate that caused the hyperuricemia increased oxidative stress; which has a potential effect on the function of cell B *in vivo* as a result of consequently a decrease in IgM and IgG levels (**Ercal, et al., (2000)**. Groups fed diets with QS / NSS or mix improved IgM and IgG levels due to seeds as an antioxidant and their superior ability to reduce internal oxidative stress. QS and *NS*S have an anti-inflammation performance to inhibit hyperuricemia from developing into gout and other inflammatory disorders. Gout takes part in many pathogenic with other inflammatory disorders, like a rapid rising in the produce of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-

α (**Terkeltaub, 2006 and Wang et al., 2015**).

CONCLUSION

This study concluded that the results showed that the mixture of QS with *NSS* improving kidney function and may significantly reduce oxidative stress and boost the antioxidant defense case.

RECOMMENDATION

The research recommended the need to supplement special meals for hyperuricemic patients and humans suffering oxidative stress by a mix of QS and *NSS* powder.

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Table (1): Elementary Chemical composition of quinoa and *Nigella sativa* seeds -dry weight- (g/100g):

Variables / Sample	Moisture	Ash	Fat	Protein%	Carbohydrates
	%				
QS	9.62	3.35	7.25	15.85	63.93
NSS	5.95	4.31	34.6	20.2	34.94

Table (2): Some minerals content of quinoa and *Nigella sativa* seeds - dry weight- (mg/100g):

Content / Sample	Zn	Fe	K	Na	Mn	Mg	Cu	Ca
	mg/100g							
QS	42.26	67.84	884.97	42.91	31.11	2533.66	17.12	**1041.66**
NSS	6.43	9.1	812.32	19.41	8.44	270.87	2.57	**590.76**

Table (3): Effect of quinoa and *Nigella sativa* seeds on biological parameters of rats suffering from hyperuricemia

Parameters Groups	BWG(g)	PI(g/d)	FI(g/d)	FER	PER
Normal control group	40.86±4.38[a]	3.01±0.12[a]	15.04±0.56[ab]	0.078±0.008[a]	0.388±0.037[a]
Hyperuricemia control group	27.03±2.31[c]	3.04±0.11[a]	14.59±0.56[b]	0.053±0.004[c]	0.255±0.027[c]
QS	33.96±1.05[b]	3.00±0.14[a]	14.62±0.39[b]	0.066±0.002[b]	0.324±0.020[b]
NSS	38.20±1.64[a]	3.06±0.12[a]	15.17±0.62[ab]	0.072±0.002[ab]	0.356±0.015[a]
QS+*NSS*	40.32±1.63[a]	3.11±0.06[a]	15.40±0.54[a]	0.075±0.005[a]	0.371±0.015[a]

Average values in every column holding unlike superscript (a, b, c) noted significant variation. Means with the same letter are insignificantly different. QS=Quinoa seed NSS= Nigella sativa seed.

Table (4): Effect of quinoa and *Nigella sativa* seeds on kidney function of rats suffering fromhyperuricemia

Parameters Groups	Urea	Uric acid	Creatinine
	mg/dl		
Normal control group	35.02±2.61[d]	3.28±0.46[c]	0.69±0.027[d]
Hyperuricemia control group	71.63±5.64[a]	8.37±1.49[a]	1.74±0.034[a]
QS	47.99±4.23[b]	5.18±0.68[b]	0.87±0.029[b]
NSS	43.54±3.93[bc]	4.22±0.14[bc]	0.75±0.030[c]
QS+*NSS*	39.75±1.55[cd]	3.61±0.25[c]	0.74±0.048[cd]

Average values in every column holding unlike superscript (a, b, c) noted significant variation. Means with the same letter are insignificantly different. QS =Quinoa seed NSS= Nigella sativa seed.

Table (5): Effect of quinoa and *Nigella sativa* seeds on total protein (T.P), albumin and globulin of rats suffering from hyperuricemia

Parameters / Groups	TP	Albumin	Globulin
	mg/dL		
Normal control group	7.98±0.35[a]	3.88±0.25[b]	4.10±0.17[a]
Hyperuricemic control group	5.28±0.15[b]	2.93±0.06[c]	2.35±0.12[c]
QS	7.77±0.55[a]	3.90±0.21[ab]	4.07±0.14[ab]
NSS	7.93±0.38[a]	4.03±0.13[ab]	3.79±0.27[ab]
QS+*NSS*	8.01±0.31[a]	3.96±0.20[ab]	4.05±0.17[ab]

Average values in every column holding unlike superscript (a, b, c) noted significant variation. Means with the same letter are insignificantly different. QS=Quinoa seed NSS= Nigella sativa seed TP= total protein

Table (6): Effect of quinoa and *Nigella sativa* seeds on SOD, GPX, GST, CAT and MDA in kidney of rats suffering from hyperuricemia

Parameters	SOD	CAT	GPX	GST	MDA
	µ/mg				**(mmol/g)**
Normal control group	94.31±4.96[b]	4.47±0.17[a]	94.87±4.28[a]	5.10±0.19[a]	6.55±0.63[c]
Hyperuricemia control group	43.94±3.04[d]	1.01±0.02[e]	30.57±1.58[d]	2.41±0.24[e]	16.36±0.72[a]
QS	87.90±7.08[bc]	2.43±0.14[d]	67.48±3.34[c]	3.28±0.18[d]	8.79±0.28[b]
NSS	82.34±5.14[c]	3.21±0.16[c]	83.48±3.56[b]	3.56±0.24[c]	8.60±0.39[b]
QS+*NSS*	102.29±4.03[a]	4.15±0.20[b]	90.71±2.26[a]	3.99±0.09[b]	6.63±0.44[c]

Average values in every column holding unlike superscript (a, b, c) noted significant variation. Means with the same letter are insignificantly different. QS=Quinoa seed NS= Nigella sativa seed

Table (7): Effect of quinoa and *Nigella sativa* seeds on HB, PCV, IgM, IgG and interleukin-1 (IL-6) of rats suffering from hyperuricemia

Parameters Groups	HB g/dl	PCV %	IgM (mg/l)	IgG (mg/l)	IL-6(pg/g) Kidney tissue
Normal	13.91±0.25^{a}	41.80±0.88^{a}	99.29±5.73^{a}	198.30±4.74^{a}	49.58±0.83^{c}
Hyperuricemia control group	10.20±0.48^{d}	30.63±1.59^{d}	54.91±3.27^{d}	117.37±5.48^{e}	83.96±5.11^{a}
QS	12.20±0.54^{b}	36.66±1.59^{b}	87.06±3.86^{c}	162.90±5.97^{d}	64.16±3.98^{b}
NSS	11.51±0.48^{c}	34.70±1.30^{c}	91.18±2.99^{bc}	170.18±5.20^{c}	60.06±3.44^{b}
QS+ *NSS*	11.97±0.27^{bc}	36.01±0.81^{bc}	95.56±3.84^{ab}	190.94±3.19^{b}	52.99±1.62^{c}

Average values in every column holding unlike superscript (a, b, c) noted significant variation. Means with the same letter are insignificantly different QS=Quinoa seed NS= Nigella sativa seed,

الدور الفعال لبذور الكينوا وحبة البركة كمضاد لزيادة حمض اليوريك في الجرذان

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الملخص العربي

أجريت الدراسة الحالية لتقييم الدور العلاجى لبذور الكينوا وحبة البركة وخليطهما علي الاصابة بارتفاع حمض اليوريك الناتجة عن تناول اوكسانات البوتاسيوم .أجري البحث على ٣٠ جرذ . قسمت هذه الجرذان الى مجموعتين أساسيتين : تركت الاولى كفئران طبيعية مجموعة ضابطة سالبة تغذت على الغذاء القياسي. المجموعة الثانية (٢٤ فأر) تمت تغذيتهم لمدة ستة اسابيع علي الوحبة الرئيسية مضاف اليها ٢٠ جرام من اوكسانات البوتاسيوم لاحداث الاصابة . ثم قسمت الجرذان الى اربع مجموعات وهى ضابطة موجبة (كنترول مريض) تغذت على الغذاء الاساسي . ومجموعات معالجة بكل من بذور الكينواأو بذور حبة البركة و مجموعة تناولت كلاهما معا. وقد كانت مدة التجربة ٣٥يوما .لقد اوضحت النتائج المتحصل عليها أنه يمكن السيطرة على ارتفاع حمض اليوريك من خلال تناول بذور الكينوا وحبة البركة فقد أظهرت تلك المجموعة أفضل النتائج الدالة على تحسن مستويات كلا من الكرياتينين واليوريا وحمض اليوريك وذلك بالانخفاض المعنوي و أيضا اسفرت عن تحسنا في مستويات الجلوبيولين والبروتين الكلى وذلك بالزيادة المعنوية. وأيضا أظهرت تحسنا معنويا في كل من الانزيمات المضادة للأكسدة بزيادة معنوية في مستويات الجلوتاثيون ترانسفيرايز والجلوتاثيون بيروكسيديز وسوبر أكسيد ديسموتيز و الكاتاليز وانخفاض معنويا في المالونالدهيد والانترلوكين – ٦وتقوية المناعة والنشاط المضاد للاكسدة مقارنة بالمجموعة الضابطة الموجبة .من خلال النتائج المتحصل عليها في هذا البحث توصي الدراسة بامكانية تدعيم الوجبات الخاصة بمرضي الكلي وخاصة ارتفاع حمض اليوريك بكلا من بذور الكينوا وحبة البركة وخليطهما لما لهما من تأثير قديحسن من وظائف الكلى ولما لها من خصائص مضادة للاكسدة وكذلك يمكن استخدام مسحوق بذور الكينوا وحبة البركة للافراد المعرضون لجهد تأكسدي.

الكلمات المفتاحية: *بذور الكينوا – بذور حبة البركة – ارتفاع حمض اليوريك*