

BENEFICIAL ROLE OF HIGH PLANT PROTEINS IN THE TREATMENT AGAINST HYPERURICEMIA IN EXPERIMENTAL RATS

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ABSTRACT

The current study was undertaken to analyze the protective effect of soybeans, white beans and fenugreek seeds on experimental hyperuricemia. Hyperuricemia was induced by potassium oxonate. Sixty adult male rats weighing 115 ± 5 gm were randomly distributed into six groups (10 animals in each group). First group served as a normal control (-ve) group, fed on standard diet only, while the other 5 groups rats were fed on standard diet containing 2% potassium oxonate for six weeks before the start of the experiment to induce hyperuricemia. Hyperuricemic rats groups were classified into control (+ve), soybeans 10% in the standard diet, white beans 10% in standard diet, soybeans 5% plus fenugreek 5% in the standard diet and white beans 5% plus fenugreek 5% in the standard diet. At the end of experimental phase for 60 days, rats were weighed then dissected and blood samples were taken. The results showed that hyperuricemic rat groups which fed on diet supplemented with soybeans plus fenugreek and white beans plus fenugreek showed non-significant difference in nutritional results compared with control group (-ve). Hyperuricemic rats fed on diet supplemented with soybeans, white beans, soybean with fenugreek and white bean with fenugreek seeds powder showed significant decrease in uric acid, creatinine, urea and nitric oxide (NO) but significant increase in total protein, albumin and globulin, antioxidant enzymes (catalase, GPX and SOD,) when compared with control (+ve) group. It is concluded that legumes increase the immunity and antioxidant activity, and the diet supplemented with two high protein legumes such as soybean with fenugreek or white bean with fenugreek had beneficial effect on nutritional and biochemical results.

Key words: Soybeans, White beans, Fenugreek Seeds, Legumes, Plant Protein, Hyperuricemia

INTRODUCTION

Uric acid is the end product of purine metabolism. Higher production of uric acid and/or lesser renal capacity to excrete uric acid leads to hyperuricemia (Ruilope and Puig, 2001). It is not only responsible for the generation of gout but also related with the occurrence of CVD renal disease, hypertension and diabetes mellitus (Feig *et al.*, 2008). It is estimated that 15 out of every 1,000 male between 35 and 45 years of age have hyperuricemia and men tends to have higher uric acid level than women (Mc Carty, 1994). It has been found that gout is most common among non-vegetarians which may be due to the presence of sulphur containing amino acids in animal protein which is responsible for excretion of acidic urine. (Kanbara *et al.*, 2010). Obesity, higher blood pressure, more animal protein and large quantities of alcohol are the other factors responsible for developing gout (Shiraishi and Une, 2009).

Many public health organizations, including the American Diabetes Association, the American Heart Association, and the American Cancer Society recommend legumes as a key food group for preventing disease and optimizing health (Ryan Borchers *et al.*, 2006). Legumes with cereals are the main plant source of proteins in human diet. Legumes contain

several phenolic compounds, in addition to glutathione, soluble proteins, and tocopherols, which are considered to be natural antioxidants (Rochfort and Panozzo, 2007). Soybeans (*Glycine max*) are unique among the legumes because they are concentrated source of flavones, dietary protein and oil throughout the world. There is also a hypothesis that flavones may inhibit atherosclerotic development, because they have antioxidant properties against LDL oxidation. Isoflavones possess hypoclesterolemic effect, due to the interaction of flavones with estrogenic receptors. The mechanism underlying the capability of plant sterols/stanols to reduce plasma LDL cholesterol levels relates to their structural similarity to cholesterol (Schmidt *et al.*, 2009). White beans (*Phaseolus Vulgarism L.*) has some bioactive components related with health benefits, such as polyphenols, lectins and carbohydrates and are good sources of folates, manganese, dietary fiber, protein, phosphorus, copper, magnesium, iron, and vitamin B₁ (Thiamin). They can lower risk of heart attack, provides energy, stabilize blood sugar, provide antioxidant benefits and help maintain the memory (Sai-Ut *et al.*, 2009). Recently, common bean is gaining growing attention as a functional or nutraceutical food, due to its rich variety of phytochemicals with potential health benefits (Heimler *et al.*, 2005). Fenugreek (*Trigonella Foenum-Greacum L.*) belongs to the plant family fabaceae or Leguminosae and

whole seeds are swallowed as anti-acid, can prevent obesity and diabetes besides lowering glucose and cholesterol (Lu *et al.*, 2008). The objective of the study is to evaluate the effect of diet supplemented with soybeans, white beans alone or combined with fenugreek on experimental hyperuricemia induced by potassium oxonate in rats.

MATERIALS AND METHODS

Materials: The experimental seeds were (soybeans, white beans and fenugreek) were purchased from the local market in Riyadh. Bio Meraux Kits were purchased from AL khan Co. for Chemicals and Biodiagnostics. The standard diet was prepared according to NRC,(1995). Potassium oxonate was purchased from El-Nil Company for inducing hyperuricemia.

Animals: Sixty Sprague Dawley strain rats weighing 120 ± 5 gm were provided by experimental Animals Center in Medicine College of King Saudi University in Riyadh. Rats were housed at a controlled temperature of $22 \pm 1^\circ\text{C}$, with 55% humidity, under 12hr light/12hr dark schedules. Animal were fed on basal diet and water was provided *ad libitum*. Rats were allowed to 7 days to adaptation before the experiment.

Preparation of Basal Diet: Basal diet consists of 140 g casein (83% protein), 100g sucrose, 50g corn oil, 50g cellulose, 35g minerals mixture, 10 g vitamin mixture, 1.8g L cystine, 2.5 g choline chloride, and the remainder (610.6 g) corn starch. Soybean, White beans, and fenugreek seeds were crushed to fine powder using a Kenwood grinder (Mainlang, China). Crude protein, crude fat, crude fiber and ash of experimental seeds were determined according to the methods of the (A.O.A.C. 2005), while total carbohydrates were estimated by subtracting the difference from initial weight of the samples as follows: $\text{Carbohydrates\%} = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ fat} + \% \text{ ash})$.

Methods: After adaptation period for one week, experimental rats were divided randomly into six groups (10 rats in each group). The first one is a normal control group (-ve) fed on standard diet only while the other 50 rats were fed on standard diet 2% potassium oxonate for six weeks before the start of the experiment containing to induce hyperuricemia as suggested by (Sharma and Sarmah, 2013). Random blood samples from orbital region of the eye were taken for estimation of uric acid. At the beginning of the study, the average weight of rat's was 115 ± 5 g and at end it increased to 185 ± 5 g. Hyperuricemic rats were classified into following groups as follows

- 1) control (+ve)
- 2) soybeans (10% soybeans in the standard diet)
- 3) white beans (10% white beans in standard diet)

- 4) soybeans with fenugreek (5% from both in standard diet)
- 5) white beans with fenugreek (5% from both in standard diet).

Daily food intake (FI) and the weekly body weight gain (WG) were recorded. Feed efficiency ratio (FER), protein intake (PI) and protein efficiency ratio (PER) was calculated according to the method suggested by (Bhilave *et al.*, 2012). At the end of experimental period (60 days) rats were fasted over night before sacrificing to obtain blood for biochemical analyses. Serum total protein, albumin uric acid, creatinine, urea, were determined as described by the method of (Schmidt, *et al.*, 2009). The experiment treatments were conducted in accordance with the ethical guidelines of the Animal Care and Use Committee of King Saud University.

Chemical analysis: Serum superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), glutathione transferase (GST) and nitric oxide (NO) were estimated according the methods described by (Kirima *et al.*, 2003) and (Wigand *et al.*, 2009) respectively, using Spectrophotometer. Hemoglobin (HG) and packed cell volume (PCV) were estimated in heparinized blood according to method described by (Hanas *et al.*, 2010) while immunoglobulin G and M (IgG and IgM) were determined by direct ELISA according to (Manohar and Selvakumaran., 2012).

Statistical evaluation of all data was carried out using the Statistical Package for Social Sciences version 11 for Windows (SPSS Inc, Chicago, IL, USA). Quantitative data are presented as means and standard deviation. The differences among the dietary treatment groups were analyzed by ANOVA; if significant differences were found, a Post-hoc analysis using LSD was performed according to (Abo-Allam, 2003).

RESULTS

Figure 1 depicts the gross chemical composition of soybeans, white beans and fenugreek. The higher levels of crude protein, fat and fiber, were found in soybean, while white bean seeds contain higher levels of ash and carbohydrate. Fenugreek seeds are rich in carbohydrate and protein. Hyperuricemic non treated group control +ve showed highly significant decrease in WG, WG %, FER and PER ($p < 0.001$) compared with control -ve group. Hyperuricemic rat groups which fed on diet supplemented with soybeans and white beans seeds powder showed significant ($p < 0.01$) decrease in WG, WG %, FER and PER compared with control group -ve but showed significant increase compared with control +ve. Food intake and protein intake were within normal values in all experimental groups. Hyperuricemia rat groups fed on soybean with fenugreek and white beans with fenugreek showed non-significant difference in WG

%, FER and PER compared with control group -ve . (Table 1).

From (table 2), it has been depicted that control +ve group had significant ($p < 0.001$) higher values of uric acid, creatinine and urea as compared with control -ve group. Hyperuricemic rat groups which fed on diet supplemented with soybeans showed significant ($p < 0.001$ and 0.05) increase in uric acid, creatinine and urea as compared with control -ve group but showed significant decrease as compared with control +ve group. Rat groups which fed on diet supplemented with soybeans with fenugreek and white beans with fenugreek showed non-significant difference in uric acid and significant ($p < 0.05$ and 0.01) increase in creatinine and urea as compared with control -ve group and significant ($p < 0.05$) decrease compared with control +ve group.

(Figure 2) illustrates that control +ve group had significant ($p < 0.01$) lower values of total protein and globulin but showed non-significant ($p > 0.05$) increase in albumin compared with control -ve group. All hyperuricemia rat groups which fed on diet supplemented with soybeans, white beans, soybeans with fenugreek and white beans with fenugreek showed non-significant ($p > 0.05$) difference in total protein, albumin and globulin as compared with control -ve group but showed significant ($p < 0.05$) increase as compared with control (+ve) group.

(Figure 3) shows that control +ve group had significant ($p < 0.001$) lower values of serum SOD, catalase, GST and GPX and significant ($p < 0.001$) higher value of NO as compared with control-ve group. Hyperuricemic rat groups fed on diet supplemented with soybeans, white beans, soybeans with fenugreek and

white beans with fenugreek seeds powder showed non-significant difference in serum SOD, catalase, and GPX compared with control -ve group but showed significant increase compared with control +ve group. They also showed non-significant difference in serum NO compared with control -ve group but showed significant decrease compared with control +ve group. Hyperuricemic rat groups fed on diet supplemented with soybeans and white beans showed significant decrease in GST compared with control -ve group and significant increase compared with control +ve group.

(Table 3) shows significant ($p < 0.01$ and $p < 0.001$) lower values of HG, PCV, Ig M and Ig G in control +ve group as compared with control -ve group. Hyperuricemic rat group fed on diet supplemented with soybeans showed significant ($p < 0.05$ and 0.01) decrease in PCV, Ig M and Ig G as compared with control -ve group but significant increase in HG, Ig M and Ig G as compared with control +ve group. Hyperuricemic rat group fed on diet supplemented with white beans showed non-significant ($p > 0.05$) difference in serum HG and PCV when compared with control -ve group but showed significant increase as compared with control +ve group. Also, this group showed significant lower value of Ig M compared with control -ve group and significant increased compared with control +ve group. Non-significant ($p > 0.01$) difference in HG, PCV, IgM and IgG observed in results were obtained from Hyperuricemic rat groups which fed on diet supplemented with soybeans with fenugreek and white beans with fenugreek seeds powder as compared with control-ve group, while significant increase as compared with control +ve group.

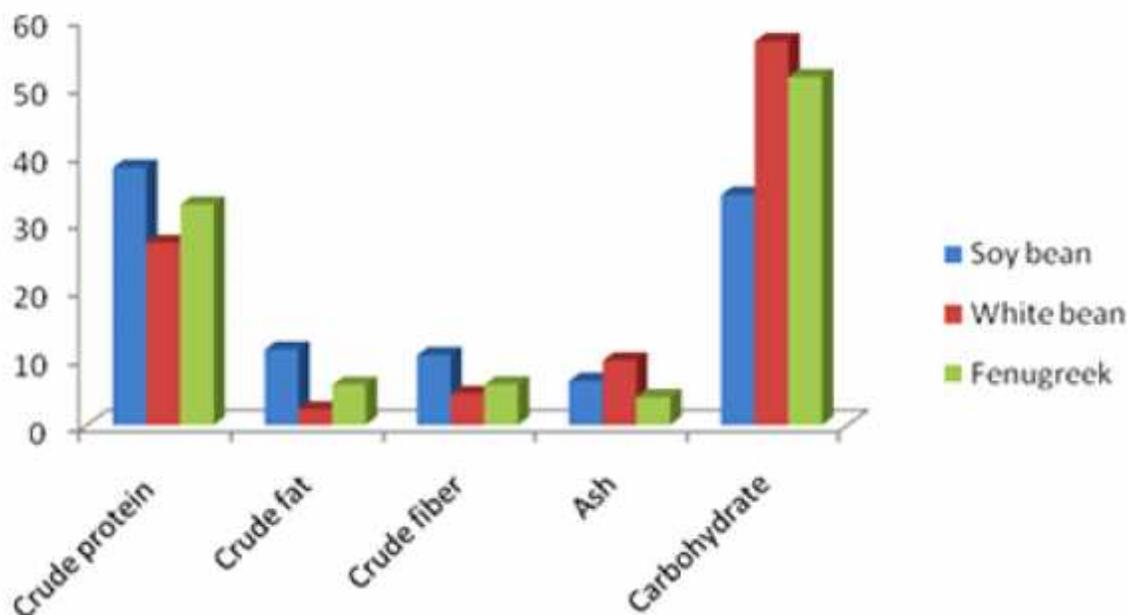


Figure 1: Gross Chemical composition of Soybean, white Bean and fenugreek (100g/dry Weight)

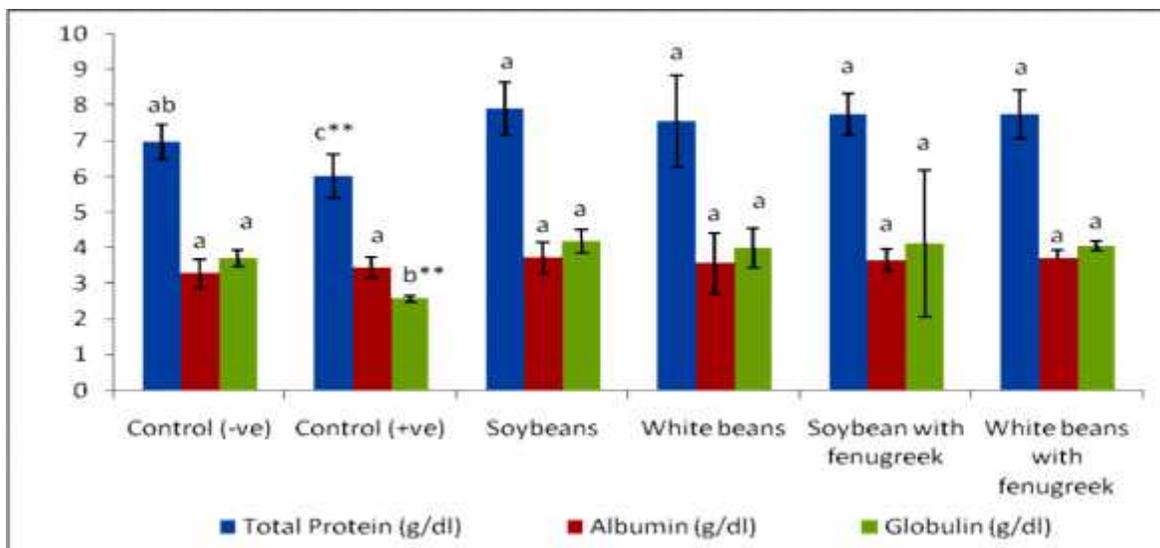


Figure 2: Mean values ± SD total protein, albumin and globulin of the experimental rat. Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001. Values with the same letters in column indicate non-significant difference (P<0.05) and vice versa.

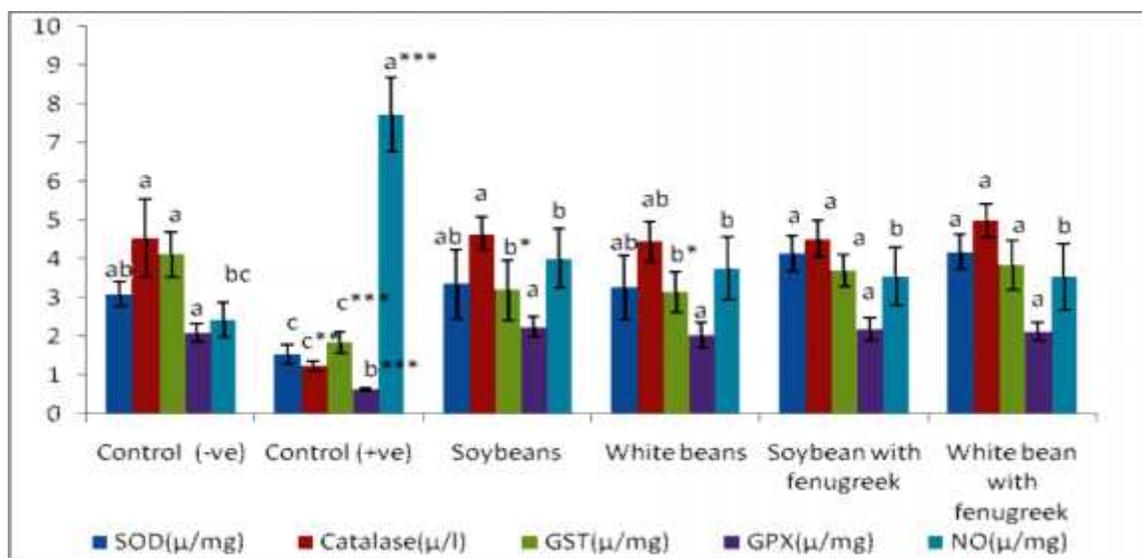


Figure 3. Mean values ± SD of serum SOD, catalase, GST, GPX, and NO, of the experimental at groups. Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001. Values with the same letters in column indicate non-significant difference (P<0.05) and vice versa. SOD-Serum superoxide dismutase, GST-Glutathione transferase, GPX- Glutathione peroxidase, NO- Nitric oxide.

Table 1. Mean values ± SD of WG, WG %, FI, FER, PI and PER of the experimental at groups.

Groups variables	WG (g)	WG%	FI (g/d)	FER	PI (g/d)	PER
Control (-ve)	87.41±3.06 ^a	53.955±2.08 ^a	16.961±1.07 ^a	0.052±0.01 ^a	3.39±0.30 ^a	0.419±0.01 ^a
Control (+ve)	47.679±1.99 ^{c***}	29.25±1.15 ^{c***}	17.22±1.09 ^a	0.028±0.002 ^{c***}	3.31±0.29 ^a	0.243±0.01 ^{d***}
Soybeans	71.14±2.78 ^{b**}	43.371±2.16 ^{b**}	17.34±1.06 ^a	0.042±0.001 ^{b**}	3.55±0.21 ^a	0.344±0.03 ^{bc**}
White beans	70.291±3.14 ^{b**}	43.38±2.9 ^{b***}	18.68±1.26 ^a	0.041±0.003 ^{b**}	3.64±0.43 ^a	0.333±0.046 ^{c**}
Soybean with fenugreek	76.73±3.50 ^{b*}	47.065±2.14 ^{ab}	17.92±1.11 ^a	0.045±0.004 ^{ab}	3.51±0.41 ^a	0.371±0.05 ^{ab}
White bean with fenugreek	80.03±3.02 ^{ab}	48.791±2.64 ^a	18.71±1.21 ^a	0.047±0.005 ^a	3.74±0.33 ^a	0.379±0.071 ^{ab}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001 Values with the same letters in column indicate non-significant difference (P<0.05) and vice versa. WG-weight gain, WG %-weight gain%, FI-food intake, FER- food efficiency ratio, PI- Protein intake and PER-Protein efficiency ratio

Table 2. Mean values \pm SD of uric acid, creatinine and urea of the experimental rat groups.

Groups variables	Uric acid (mg/d)	Creatinine (mg/d)	Urea(μ /mg)
Control (-ve)	2.49 \pm 0.329 ^c	0.683 \pm 0.039 ^d	19.70 \pm 1.71 ^d
Control (+ve)	8.511 \pm 1.102 ^{***}	1.747 \pm 0.212 ^{a***}	52.911 \pm 4.079 ^{a***}
Soybeans	4.382 \pm 0. 522 ^{**}	0.929 \pm 0.0145 ^{b*}	32.007 \pm 4.611 ^{b**}
White beans	4.193 \pm 1.494 ^{**}	0.915 \pm 0.043 ^{b*}	30.306 \pm 3.22 ^{b**}
Soybean with fenugreek	3.174 \pm 0. 507 ^{bc}	0.808 \pm 0.029 ^{c*}	28.405 \pm 2.68 ^{b**}
White bean with fenugreek	3.215 \pm 0. 0.29 ^{bc}	0.806 \pm 0.021 ^{c*}	72.329 \pm 2.905 ^{bc*}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001. Values with the same letters in column indicate non- significant difference (P<0.05) and vice versa

Table 3. Mean values \pm SD of HG, PCV, IgM and IgG of the experimental rat groups.

Groups variables	HG	PCV	IgM (mg/l)	IgG (mg/l)
Control (-ve)	12.47 \pm 1.07 ^a	37.739 \pm 3.31 ^a	98.947 \pm 8.347 ^a	197.94 \pm 22.75 ^a
Control (+ve)	10.55 \pm 1.16 ^{b**}	29.30 \pm 2.09 ^{c***}	64.41 \pm 5.93 ^{c***}	128.15 \pm 9.78 ^{c**}
Soybean	11.608 \pm 1.26 ^a	34.00 \pm 3.06 ^{bc*}	88.334 \pm 8.56 ^{b*}	160.56 \pm 12.71 ^{cd**}
White bean	11.472 \pm 0.417 ^a	33.84 3.329 ^{ab}	89.634 \pm 8.704 ^{b*}	164.499 \pm 14.17 ^{c*}
Soybean with fenugreek	11.05 \pm 1.268 ^a	4.21 \pm 3.116 ^{ab}	96.709 \pm 9.11 ^a	187.50 \pm 14.70 ^a
White bean with fenugreek	11.423 \pm 1.30 ^a	35.90 \pm 3.314 ^a	100.48 \pm 8.737 ^a	186.659 \pm 14.3 ^{ab}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001. Values with the same letters in column indicate non- significant difference (P<0.05) and vice versa.

HG–Hemoglobin, PCV- Packed Cell Volume, Ig M– Immunoglobulin M, Ig G- Immunoglobulin G

DISCUSSION

Uric acid (UA) is a constituent of the cell cytosol and also one of the soluble compounds in the blood. It is mainly synthesized from adenine- and guanine-based purines. Uric acid may be found in all tissue compartments except in those of the lipid phase (Nasar *et al.*, 2015). Hyperuricemia is an excess of uric acid in the blood. Uric acid passes through the liver, and enters blood stream. The normal uric acid levels are 2.4-6.0 mg/dl (female) and 3.4-7.0 mg/dl (male). This high uric acid levels in the blood may cause problems, such as kidney stones or kidney failure, and gout i.e. collection of uric acid crystals in the joints, especially in toes and fingers. Purines are nitrogen-containing compounds, which are made inside the cells of the body (endogenous), or come from outside from foods containing purine (exogenous). Purines break down into uric acid, and increase the levels of uric acid. Purines may accumulate in the tissues, and form crystals (Kaneko *et al.*, 2014)

The results (Fig.1) of gross composition of soybeans powder have higher levels of crude protein, fat and fiber and this is in agreement with USDA (2009). Ash and carbohydrate was found to be highest in white bean and these values were quiet similar to the result obtained by (Thompson *et al.*, 2009). White bean analysis shows that the carbohydrates represent is a major component. It includes soluble and insoluble dietary fiber as well as probiotics oligosaccharides (Murquiz *et al.*, 2012) and (Vidal-Valverde *et al.*, 2002) Fenugreek seeds

were found to be rich in carbohydrate and protein. This result is similar to those obtained by (Elmnan *et al.*, 2012).The proximate composition of many legumes have been widely documented in many literature, and the data are not always comparable due to the different of analysis methods genotypes, environments, cooking and de hulling process. (XU and Chang, 2008 and Wang and daun, 2006)

Potassium oxonate is uricase inhibitor associated with renal insufficiency and used for inducing chronic insufficiency. The oxonate-treated rat can serve as a useful animal model not only in investigation of the uric acid nephropathy, but also in a number of other toxicological evaluations connected with uric acid (Al Tamim, 2014 and Wang *et al.*, 2015)

Body weight and food efficiency results suggest that hyperuricemia may prevent an increase in body weight and low body weight as compared to control negative in hyperuricemic and treated groups may be partially due to the loss of appetite. Hyperuricemic rat groups which fed on diet supplemented with soybeans and white beans seeds powder showed increase in WG, WG%, FER, and PER, compared with control +ve . This result was partially agreed with that reported by (Kim *et al.*, 2012) and (Al Ahdab. 2014) who found that soy protein isolate caused improvement in body weight gain and reduction in adipose tissue weight by affecting the activities of hepatic lipogenic enzymes in rats fed on high-fat diet. White bean results also were in agreement with (Heimler *et al.*, 2005) and (Sai- Ut *et al.*, 2009) who reported that white bean are good source of protein

dietary fiber, lipids and it is in demand due to its rich phytochemicals potential health benefits. However, little is known about their photochemistry and antioxidant activity (LU *et al.*, 2008) and (Wang and Daun, 2006). Urea and ammonia are the end products of protein metabolism and arises from the partial oxidation of amino acids. Uric acid and creatinine are indirectly derived from amino acids as well. Protein deficiency has also been shown to adversely affect kidney function, where it has adverse effects on both glomerular and tubular function. In addition, urinary creatinine excretion has been used as a reflection of muscle mass (Corish and Kennedy., 2000). In this study increase in urea, uric acid and creatinine was observed which might be caused as result of metabolic disorder (Table 2). In a study it has been described that in diabetes glycolization of protein causes high demolition of muscles and increase of purine level, which is the main source of uric acid (Sauvaire *et al.*, 1998). Kaneko *et al.*, 2014 reported that the dried soybean have higher levels of purine and uric acid (172.5 mg and 201.7mg /100g), while broad bean have 35.5mg,41.5mg/100g and green peas have 18.9mg and 21.9mg/100g respectively.

Kidneys maintain optimum chemical composition of body fluids by acidification of urine and removal of metabolite wastes such as urea, uric acid and creatinine. In renal disease, the concentrations of these metabolites increase in blood (Chandramohan, 2009). The results indicated that, as compared with control -ve group, soybean isoflavones, white bean Polyphenols and folates in combination with fenugreek significantly reduced serum uric acid, creatinine and urea (Xu *et al.*, 2008). In study of (Yu Sheng , 2013), the result shows that the anti-hyperuricemic effect of soybean isoflavones were attributed to uric acid excretion enhancement. Messina *et al.*, (2011) conducted a review of soy foods and hyperuricemia and found no evidence of increased uricemia related to soy intake.

Uric Acid (UA) is the final product of the activity of xanthine oxidase (XO) in the purine metabolism. The enzyme, XO linked to oxidative stress, endothelial dysfunction and heart failure in humans. Clinically, an association of hyperuricemia with hypertension, diabetes, renal disease and cardiovascular disease (CVD) has been observed over the years. Serum urates have been associated with many cardiovascular risk factors including aging, obesity, hyperlipidemia, renal disease and insulin resistant syndrome (Ruilope and Puig, 2001). An increased level of uric acid also increases morbidity and mortality (Akram *et al.*, 2011).

Legumes consumption can reduce the risk of metabolic syndrome because of such component as soluble fiber, complex carbohydrate, vitamins, minerals and phytoestrogen especially isoflavones, genistein, daidzein, and glycitein (Jenkins *et al.*, 2002). In a study on the consumption of oat bran, soy protein, and flax seed for three months although did not reduce the lipid levels

of the patients but it did not contribute to an increase in hyperuricemia (Ferreira *et al.*, 2013). The levels of plasma total proteins were found to be decreased in this study. This could be due to increased lipid peroxidation. Protein synthesis is decreased in all tissues due to decreased production of ATP in absolute or relative deficiency of insulin (Chatterjee *et al.*, 1994). This may be responsible for the decreased level of plasma proteins in hyperuricemic rats. Uric acid is tightly bound to γ -globulin, although only in small quantities (about 0.1-0.2 mg/dl). Other protein binding is considered to be loose, thus permitting glomerular filtration of uric acid. Previous studies suggests that albumin is one determinant of uric acid levels and is supported by in vitro studies indicating 1 gm/dl of albumin will bind 0.6 mg/dl of uric acid (Adams *et al.*, 2005).

Antioxidants constitute the primary defense system that restricts the toxicity related with free radicals. The equilibrium between antioxidants and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles. Rats pretreated with beans and fenugreek showed increased activities of these enzymes which suggest that they have the ability to prevent the deleterious effects induced by free radical. (Naidu *et al.*, 2011). Increase in the activities of GPX and GST in treated group shows the antioxidant potential of beans and fenugreek against injury caused by free radicals. Free radical scavenging enzymes such as SOD, catalase, GPX, and GST are the first line of cellular defense against oxidative injury. Lowering of enzymes might be due to enhanced lipid peroxidation (Rahmathulla 2013).

Conclusion: The results showed that hyperuricemic rat groups which fed on diet supplemented with soybeans plus fenugreek and white beans plus fenugreek showed non-significant difference in nutritional results compared with control group -ve. Hyperuricemic rat groups which fed on diet supplemented with soybeans, white beans, soybean with fenugreek and white bean with fenugreek seeds powder showed significant decrease in uric acid, creatinine, urea and nitric oxide (NO) but significant increase in total protein, albumin and globulin, antioxidant enzymes (SOD, catalase, and GPX) when compared with control +ve group. It is concluded that diet supplemented with two high protein legumes such as soybean with fenugreek or white bean with fenugreek had beneficial effect on nutritional and biochemical results. Legumes are the main plant source of proteins in human diet, and they are generally rich in dietary fiber and carbohydrates. Legumes contain several phenolic compounds, in addition to glutathione, soluble proteins, and tocopherols, which are considered to be natural antioxidants. So, still more studies are needed to investigate the effect of different cooking methods on the acceptability and bioavailability of these legumes.

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REFERENCES

- A.O.A.C. (2005). Official methods for analysis of the association of official analytical chemist. A. O.A.C., 12th Ed. Washington, D.C.
- Abo-Allam, R.M. (2003). Data statistical analysis using SPSS program. 1st Ed. Cairo.
- Adams, W.H., J.A. Harper, P.M. Heotis, and A.H. Jamner (1984). Hyperuricemia in the inhabitants of the Marshall Islands. *Arthritis and Rheum.* 27: 713-716.
- Akram, M., H. M. Asif, K. Usmanghani, N. Akhtar, Q. Jabeen, A. Madni, T. Saeed, R. Rehman, K. Ahmed, and S.M. Ali Shah (2011). Obesity and the risk of hyperuricemia in Gadap Town, Karachi. *Afr. J. Bio technol.* 10: 996-998.
- Al-Ahdab, M.A.(2014). Beneficial effects of substitution of vegetable soy protein instead of animal protein casein in diets for diabetic male rats. *Nat. Sci:* 12: 120-128.
- Al-Tamim, E.A.A. (2014). Effect of cake fortified with pineapple consumption on hyperuricemic rats. *Curr. Sci. Int.* 32: 71-77.
- Bhilave, M.P., S.V. Bhosale, and S.B. Nadaf (2012). Protein Efficiency Ratio of Ctenopharenged on idella Fed on soybean formulated feed. *Biological forum. Inter . J.* 4: 79-81.
- Chandramohan, G., K.S. Al-Numair, and K.V. Pugalendi (2009). Effect of 3-hydroxymethyl xylitol on hepatic and renal functional markers and protein levels in streptozotocin diabetic rats. *Afr. J. Biochem. Res.* 3:198-204.
- Chatterjee, M.N., R. Shinde (1994). Text Book of Medical Biochemistry. 8th Ed. Jaypee Brothers Medical Publishers Pvt Ltd; New Delhi (India). pp. 1-876.
- Corish, C. A., and N.P. Kennedy (2000). Protein energy under nutrition in hospital in-patients. *Br .J. Nutr.* 83:575-591.
- Elmnan A., A. Balgees, and J.L. Mangara (2012). Effect of fenugreek (*Trigonella foenm greacum*) seed dietary levels on lipid profile and body weight gain of rats. *Pakistan J. Nutr.* 11: 1004-1008.
- Feig, D.I., D. H. Kang and R. J. Johnson (2008). Uric acid and cardiovascular risk. *N. Eng. J. Med.* 359: 1811-1821.
- Ferreira, Rdos. S., D.C. Cassaro, H. Domingos, E.R. Pontes, P.H. Aiko, and J.E. Meira (2013). The effects of a diet formulation with oats, soybeans, and flax on lipid profiles and uricemia in patients with AIDS and dyslipidemia. *Rev. Soc. Bras. Med. Trop.* 46:691-697.
- Hanas, R., and G. John (2010). Consensus statement on the worldwide standardization of hemoglobin A1C measurement. *Daibetes Care.* 33:1903-1904.
- Heimler, D., P. Vignolini, M.G Dini, and A. Romani(2005). Rapid tests to assess the antioxidant activity of *Phaseolus vulgaris* L.dry beans. *J. Agric. Food. Chem.* 53: 3053-3056.
- Jenkins, D.J., C.W. Kendall, C.J. Jackson, P.W. Connelly, T. Parker, D. Faulkner, E. Vidgen, S. C. Cunnane, L.A. Leiter, and R.G. Josse (2002). Effects of high and low isoflavone soya foods on blood lipids, oxidized LDL, homocysteine and blood pressure in hyperlipidemic men and women. *Am. J. Clin. Nutr.* 76: 365-372.
- Kanbara, A., M. Hakoda, I. Seyama (2010).Urine alkalization facilitates uric acid excretion. *Nutr J.* 9: 45.
- Kaneko, K., Y. Aoyagi, T. Fukuuchi, K. Inazawa, and N. Yamaoka (2014). Total purine and purine base content of common foodstuffs for facilitating nutritional therapy for gout and hyperuricemia. *Biol. Pharm. Bull.* 37:709-721.
- Kim, J., H.J. Lee., J.Y. Kim, M.K. Kim and O. Kwon (2012). Plant proteins differently affect body fat reduction in high-fat fed rats. *Prev. Nut. Food Sci.* 17: 223-227.
- Kirima, K., K. Tsuchiya, H. Sei, T. Hasegawa, M. Shikishima, Y. Motobayashi, K. Morita, M. Yoshizumi and T. Tamaki (2003). Evaluation of systemic blood(NO) dynamics by EPR Spectroscopy: HbNO as an endogenous index of NO. *Am.J. Physiol. Heart Circ. Physiol.* 285: 589-596.
- Lu, F.R., L. Shen, Y. Qin, L. Gao, H. Li, and Y. Dai (2008). Clinical observation on *trigonella foenum-graecum* L. total saponins in combination with sulfonylureas in the treatment of type 2 diabetes mellitus. *Chinese J .Inter. Med.*14:56-60.
- Manohars, S., and R. Selvakumaran(2012). Estimation of serum immunoglobulin E (IgE) level in allergic asthma and allergic rhinitis patients before and after treatment. *Euro. J. Experiment Biol.* 2: 2199-2205.
- McCarty, D.J. (1994). Gout without hyperuricemia. *J A.M.A.* 271: 302-304.
- Messina, M.,V.L. Messina, and P. Chan (2011).Soy foods, hyperuricemia and gout: a review on the epidemiologic and clinical data. *Asia Pac. J. Clin. Nutr.* 20: 347-358.

- Murquiz, M., A. Varela, C. Burbano, C. Cuadrado, E. Guillmon, and M.M. Pedrosa (2012). Bioactive compounds in legumes: pronutritive and antinutritive actions. Implications for nutrition and health. *Photochem. Rev.* 9: 227-244
- Naidu, M.M., B.N. Shyamala, J.P. Naik, G. Sulochanamma, and P.Srinivas(2011). Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. *LWT – Food. Sci. Technol.* 44: 451-456.
- Nasar, Md. A.,T.F. Subhani, R. P. Gupta, A. Naseer, S.S. Subhani (2015). Uric acid levels in serum in the assessment of some psychiatric disorders in India. *J. Med. Radiol .Pathol. Surg.* 1: 9–12.
- NRC (1995). National Research council: Nutrient requirements of laboratory animals. 4th revised Ed. National Academy Press; Washington, DC. pp:29-30.
- Rahmathulla, S.B.M. (2013). Effect of Tribulus terrestris fruit aqueous extract on hyperlipidemia and maintenance of liver architecture in isoproterenol-induced myocardial infarction in rats. *Int. J. Pharm. Bio. Sci.* 4: 983 – 992.
- Rochfort, S., and J. Panozzo (2007). Phytochemicals for health, the role of pulses. *J. Agri. Food Chem.* 55:7981–7994.
- Ruilope, L.M., and J. Garcia Puig (2001). Hyperuricemia and Renal function. *Curr. Hypertens. Rep.* 3: 197-202.
- Ryan Borchers, T.A., J.S. Park, B.P. Chew, M.K .McGuire, L.R. Fournier, and K.A. Beerman (2006). Soy isoflavones modulate immune function in healthy postmenopausal women. *Am. J. Clin . Nutr.* 83:1118-1125.
- Sai-Ut, S., S. Ketnawa., P. Chaiwut and S. Rawdkuen (2009). Biochemical and functional properties of proteins from red kidney, navy and adzuki beans. *As. J. Food Ag-Ind.* 2: 493-504.
- Sauvaire, Y., P. Petit, C. Broca, M. Manteghetti, Y. Baissac, J. Fernandez-Alvarez, R. Gross, M.Roye, A. Leconte, R. Gomis, and G. Ribs (1998). 4- Hydroxy Isoleucine: A novel amino acid potentiator of insulin secretion. *Diabetes.* 47:206-210.
- Schmidt, E.M dos S., A.C. Paulilo, I.M. Lopera, G.R.V. Martins, L.N. Junio, A.J.P. Testi. J. Denadi, E.J.L. Hoppe, and J.J. Faqliari (2009). Evaluation of serum biochemical parameters in Juvenile bronze Turkeys. *Int. J. Poul. Sci.* 8:746-748.
- Sharma, B., and D. Sarmah(2013). Comparative evaluation of sodium and potassium measurements by flame photometer and by direct ISE methods. *Int.J.Health Sci.Res.* 3: 59-65.
- Shiraishi, H., Une. H. (2009). The effect of the interaction between obesity and drinking on hyperuricemia in Japanese male office workers. *J. Epidemiol.*19: 12-14
- Thompson, M.D., M.A. Brick., J.N. McGinley, and H.J. Thompson (2009). Chemical composition and mammary cancer inhibitory activity of dry bean. *Crop.Sci.* 49:179-186.
- USDA (2009). National Nutrient Database for Standard Reference, Release 19. Accessed on 26th April, 2015. www.usda.gov.
- Vidal-Valverde, C., J. Frias., I. Sierra, I. Blazquez, F. Lambein, and Y.H Kuo (2002). New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. *Euro. Food Res. Technol.* 215:472-477.
- Wang, MX. Y.L.Y yang., Y.Yang. D.M. Zhang, and L.D. Kong (2015).Nuciferine restores potassium oxonate, induced hyperuricemia and kidney inflammation in mice. *Eur. J. Pharmacol.* 747: 59-70.
- Wang, N. and J.K. Daun (2006). Effects of variety and crude protein content on nutrient and anti-nutrients in dried green peas (*Len culinaris*, L.). *Food Chem.* 95: 493–502.
- Wigand, H., A.E. Wagner, C. Boesch-Saadatmandi, H.P. Kruse, S. Kulling, and G. Rimbach (2009). Effect of Dietary Genistein on phase I and anti oxidant enzymes in rat liver. *Cancer. Genomics and Proteomics.* 62: 85- 92.
- Xu, B., and S.K.C. Chang (2008). Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *FoodChem.*110: 1-13.
- Yu-Sheng,C.(2013). Study on Anti-hyperuricemia activity of soybean isoflavone soybean. *Soyabean. Sci.*279-282.