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PROTECTIVE EFFECT OF LEGUME PROTEINS IN TREATMENT OF HIGH LEVELS OF BLOOD URIC ACID IN HYPERURICEMIC RATS

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ABSTRACT

The research was conducted to estimate the chemical composition of plant legumes, which are highly protein such as Indian fenugreek, white lupine seeds and yellow lentil, seeds The study also was evaluated of the protective effects of feeding legume seeds supplement diets on experimental Hyperuricemic rats. The supplemented diet on Potassium oxonate 2% were increased blood level of uric acid, which caused Hyperuricemia . Sixty different male adult rats randomly weighing 160±8. were separated to six groups: One group were consisted of 10 rats serving as a control negative group (–ve), were feeding on regular diet only, at the same time the fifty others separated per fife groups, (ten rats in each one) feed them same intake contains 2% of potassium oxonate for 6 weeks earlier than starting the experiment. The Hyperuricemia groups were divided to:- 1- control group +v (fed stander diet only) 2- white lupine group (which fed stander diet plus 10% white lupine seed powder). 3- yellow lentil group (which fed 10% yellow lentil in average diet), 4- (white lupine seed 5% plus 5% dried leaves fenugreek powder in standard diet group) 5- (yellow lentil 5% plus 5% powder of fenugreek seeds in standard diet group.) After two months Hyperuricemic rats which feed scheduled diet supplement.

Potassium oxonate 2% were sacrificed to taking blood samples for analysis. The results observed that the control rat groups –ve nonsignificant differences in nutritional result compared with Hyperuricemic rat groups which fed on diet addition fenugreek with white lupine and lentil. Hyperuricemic rats which treatment with legumes seed powder (white lupine, lentil and the mixer with fenugreek) were recorded significantly decreased in urea, uric acid, creatinine and (NO) nitric oxide, while resulting significantly increased in albumin globulin, total protein, antioxidant enzymes as SOD, Catalase, GPX and Immunoglobulin (Ig M and Ig G), in compared with control positive group (+ve). The conclusion of this search prove that the diet added the two rich protein-legume such as white lupine with a fenugreek or lentil with the fenugreek, had an enhancement effect on nutritional dietary, biochemical analysis. and increases the immunity

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per antioxidant activity. thus So its considered good treatment to high uric acid, purines, and Hyperuricemia, which are causes of gout.

Keywords: Protective Effect, Legume, Hyperuricemic Rats, Protein, Biological Effects

1. INTRODUCTION

The main structure of body cells, and major for all functions is protein. It's important to keep body in good condition, for healthy tissues, physical structure of body, including the bones, flesh, and organs proteins, amino acids, enzymes, membrane serum albumin, collagen, keratin, blood molecules and hormones. The essential amino acids of protein are important for effectives performance of many essential function of nucleic acid coenzymes, hormones and molecules fundamental for life (Kim; et al; 2012)

Legume seeds are a good sources of plant protein, carbohydrate, fat, minerals and dietary fibre. Its content more 20- 25% protein 2-3 times higher the content in cereals. Legumes are the main plant source of proteins in human diet. Although the legume high content of protein there are deficiency in essential amino acids and the biological effects, compared with meat proteins. (Sahar and Ashraf; 2008). Legumes are antioxidants contain tocopherols, phenolic compounds, soluble proteins and glutathione enzyme (Rochfort and Panozzo; 2007. legumes are the beneficial food for preventing many chronic diseases like diabetes kidney disease gout and obesity (Cecilia et al 2018) Its observed recently that the legumes are higher of phytochemicals variability with likely health assistances so, achievement increasing kindness of nutraceutical food or functions, White Lupine (Lupines Albus) (Sweet lupine) is good legume in human and animal food owing to the high protein content (85% globulins and 15% albumins) (Martinez-Villalluenga et al., 2009). It is recognized as sweetened lupines to their little amount of bitter-tasting and possibly poisonous alkaloids. Though, for excessive alkaloid content, the seeds could not be reflected as a safe food component The importance function of white lupine component to be decreasing disorder being form, as well as obesity, lipid concentration, colorectal, cardiovascular diseases, insulin struggle diabetes, glycaemia, appetite, hypertension, and cancer concern. (Janusz Prusinski., 2017). Seeds are used to produce gluten-free proposal assistances antioxidant and support preserve the celebration reduced dangerous of heart attack, provides energy and pry diabetes, (Sai-Ut tal., 2009. Lentil (Lens Culinaris L) legume have necessary essential components of the human diet ,due to its have rich of proteins, bioactive peptides, phytochemicals and antioxidants (Mitchell et al., 2009). Some revisions have proved that the assimilation of lentil is immensely associated to lessening of illness as diabetes, obesity, cancers and cardiac diseases faithfully to its bioactive compounds. Scientific increasing discuss attention to lentils as the functional food residual to nutritive values of compounds activity and

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polyphenols (Kumar and Baojun, 2017). The plants sterols/stanols is able to reduce plasma LDL cholesterol levels relays to cholesterol structural relationship. (Schmidt. et al., 2009). (Malia, Frey; 2018) Fenugreek (trigonella Foenum greacum). goes to the leguminosae whole seeds plant family and are accepted as antiacid, can inhibit obesity and diabetes too lower in cholesterol and blood glucose (Lu et al., 2008 and). Fenugreek Powdered has a golden yellow colour in line for its colouring agent called Coumadin. Seeds of fenugreek have several effective biological substances might be powerful therapeutic supplement. and nutraceutical. (Jadwiga. rystyna 2017). Fenugreek seeds are acidic, has a powerful and very specific odour, so consumed in a very little amount. fenugreek has documented as healthy food taken as supplements, root vegetable, or medicinal remedies. (Kassiani et al., 2009)

Uric acid is the major etiological factor of gout. It is the product endogenous of purine metabolism. (Jessica. M, et al; 2016). (Feig et al., 2008) observed that more of uric acid reduced renal ability to eject uric acid reasons to Hyperuricemia. The other factors reliable to intensifying gout such as renal diseases , diabetes mellitus, hypertension, obesity, high blood pressure, and more consumption of proteins (Shiraishi and Une, 2009). and (Mamada, C; and Galanis N; 2018). Uric acid is higher in men than women among 35 to 45 years of age (Almasri. S, 2014). Kanbara et al., 2010) were established that the gout is most normal in non-vegetarians could be to the existence of sulpher amino acids furthermore protein dependable for emission of urine acidic (The major site of uric acid creation is the liver be caused by of the failure of nutritive purines and purine combinations created by form (Axel F. and Sigurdsson MD 2018) Normal uric acid levels are 3.4-7.0 mg/dl for males and 2.4-6.0 mg/dl of females. Purines are the nitrogen foods breaks to uric acid can be increased accumulate in connective tissues, producing crystals in joints especially in toes and fingers, when the blood uric acid level overhead 7 mg/dl. caused problems, like kidney stones, and gout (Almasri; 2017). Hyperuricemia may be main provider to the evolution or advancement of chronic kidney disease (CKD). Present it is not obvious that reduction of uric acid (UA) was attached to the danger for kidney destruction, it seems that increased on UA danger arises. Lifestyle associations, with exercise, lessening weight, low intake of meat higher in purine- or avoiding too much fructose consumption are suggested for all Hyperuricemia patients (Ramirez and Madero 2018) uricemia is an extra of uric acid in the blood, passes about the liver, and inserts your blood stream.

So, the aim of research were analysis the chemical structure of seed legumes high plant protein, and revision the consequence of consume white lupines and lentil alone or combined with fenugreek diet additions in medication the higher of uric acid, and hyperuricemia that cause gout and kidney failed.

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2. MATERIALS AND METHODS

Plant Materials

White lupines (Lupines Albus), lentil (Lens Culinaris L) and Indian fenugreek (Trigonella Foenum greacum) were purchased from the Riyadh market. Casein, cellulose, starch ,vitamins and the Bio-Meraux Kits were obtained from Alkan Saudi chemical Co. for Chemical analysis and Bio-diagnostics'.

Experimental Animals

Sixty male albino rats (Sprague Dawley strain) weighing 160 ± 8 g. were carried out from animals house of Medicine collage, King Saudi University. Rats were qualified one week to acclimatization previously the experiment. It's were adjusted at a measured degrees $21\pm^{1o}$C, under 55% humidity, at 12 hr light /and 12 hr. in dark overnight schedules.

METHODS

Preparation Stander Diet

The regular diet was structured in agreement with NRC (1995). Its consists of (cornoil 50g), (casein 83% protein 140g), (sucrose 100g) (cellulose 50g), (mixture minerals 35g) vitamin mixture 10g) (L cystine 1.8g), (2.5 g chloride choline), and the balance (corn starch. 610.6 g), White lupine Lentil and Indian fenugreek seeds were grinding to suitable powder by Kenwood grinder (Mabinlang, China). Approximate crudes of (fat, protein, fiber) and ash. Seeds of experimental were analysis agreeing to the procedures of the A.O.A.C. (2016) Carbohydrates% =100- (% moisture+ % protein+ %fat +%ash) were estimated as follows of (Stefanie and Live 2015)

After (one week) the adaptation rats were divided into negative control group 10 rat fed usual diet only, however the others 50 rats were consumed on regular quantity having 2% Potassium oxonate to inducing hyperuricemia, for six weeks earlier the experiment according to previous study, by (Sharma and Sharma, 2013.) At the end of experimental period (60 days), rats were weighing 190±5 g. Rats had Hyperuricemia were systematic into Fife groups as follow:

1- control (+ve) which fed normal diet

2- lupines group (fed standard diet plus white lupine10%),

3- lentil group (fed standard diet with 10% lentil powder)

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4- white lupines with Indian fenugreek seeds(normal diet add to 5% from each one) 5 - lentil with Indian fenugreek seeds(regular diet combine with 5% from both).

Chemical Analysis:

After 2 months, the rats fasted over night before killed to obtaining blood samples. Part of blood was centrifuged to obtain serum for biochemical analyses of uric acid, urea, total protein, creatinine and albumin in similarity with Henry (2001). (Bhilave et al 2012) were explained the techniques of analyze blood serum. Liver Superoxide dismutase (SOD), glutathione peroxidase, GPX), Catalase Enzyme, Nitric Oxide (NO) were logical by Enzymatic Colorimetric Procedures, but (HG) Hemoglobin and (PCV) Packed Cell Volume were measured in Heparinized Blood while, immune globulins (IgG & IgM) were calculated by the enzyme-linked immunosorbent assay (ELISA) in agreement with (Hanas et al 2010) and (Manohar and Selvakumaran 2012) respectively.

Statistical Analysis

All obtained statistics data were statistically analyzed evaluated one way analysis of variance. (ANOVA) was used to test the differences between groups pursued LSD tested by computer software and SPSS agreeing with Abo-Allam (2003).

3. RESULTS

Data in figure 1 showed that protein, fat and fiber were richer in seeds of white lupines than lentils seeds which have greater on ash and carbohydrate, but Fenugreek seeds were recorded higher in carbohydrate and protein. Hyperuricemic rats positive control (+ve), white lupines and lentil seeds groups, had significant decreased in WG, WG %, FER and PER at P 0.001 equaled with control-ve group, but presented significantly increase with control (+ve) group.

Values of food intake and protein intake were in average in all groups. Rats Hyperuricemic group which consumed of diet added white lupines plus fenugreek and lentil with fenugreek seeds were noticed none significantly variances in WG %, FER and PER measured by negative control(-ve) group as presented in table 2

In table 3 its appeared that the control (+ve) group resulted significantly elevated values at P 0.001 in uric acid, creatinine and urea groups when related with control group (-ve). Rats Hyperuricemic group supplied intake by way of white lupines and lentil seeds powder indicated that significant increased and significant decreased had in uric acid, creatinine and urea at P 0.001 & P 0.05 in compared with negative control (-ve) and positive control (+ve) groups

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respectively. Hyperuricemic Groups which supported by lupines plus fenugreek seeds or lentil with fenugreek seeds were caused non-significant variances in uric acid concentration and significant heighten creatinine and urea levels at p 0.05 & 0.01 as related to group negative control (-ve), while its decreased significant related to the positive control group (+ve).

IN Table 4 significantly lower values of total protein and globulin at p 0.01 illustrated in control (+ve) group, on the other hand non- significant increases in albumin as paralleled with negative control group (-ve). All rats with Hyperuricemia served improved diet using seeds of white lupines, lentil, white lupines in addition to fenugreek and lentil per fenugreek suggested that nonsignificant changes at p 0.05 in total protein, albumin and globulin contrasting with -ve group control however in positive group (+ve) were recorded significantly increased Data in table 5 suggested that significant lower values of serum SOD, catalase, GST and GPX at P 0.001 and significant higher value of NO with (+ve) control group in comparing (-ve) group, Its observed non-significant differences in the similar measures with Hyperuricemic rats consumed diet including all treated seeds powder measured by the negative control group (-ve). while recoded significantly increased per (+ve) control group. It's also pointed insignificant variances in (NO) serum contrasted with negative group control (ve) although indicated reduced with control +ve group. Significant decreased in GST noticed when Hyperuricemic rats were given white lupines and lentil on usual diet with compared by (-ve) group control although the (+ve) group control carried to significantly increased.

In figure 6 Data recommended significant lower on evaluates of HG, PCV, Ig M and Ig G at P 0.01 & P 0.001 in control group (+ve) when compared with control (-ve) group. Rats of Hyperuricemic indicated significant decrease in these values at P 0.05 & P 0.01 when nourished diet with white lupines. compared with (-ve) control group, while the significances of HG, Ig M and Ig G. were increased in (+ve) group control. So lentil powder supplementation on same diet feeding by Hyperuricemic rats recommended none significantly modification of HG, PCV, and IgG serum blood at P 0.05 rivalled with (-ve) control group nevertheless revealed significant increase compared with (+ve) control group. Similarly group revealed same significant lower and significantly increase values of Ig M when estimated by control (-ve) group and control +ve group, respectively Diet complemented powder of lupines together fenugreek as well as lentil plus fenugreek seeds cleared non significantly differentiation and significant increase on standards of PCV, HG, Ig G and Ig M at P 0.01 compared with (-ve) and (+ve) control groups.

4. DISCUSSION

White lupines, lentil and fenugreek have Chemical composition expected in many texts however statistics not equal because the variances in conditions, genotypes, cooked , duelling

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methods and techniques of evaluation (Wang et al., 2009). The attained consequences of crud structure of lentil seeds powder remained approved by (Costa et al. 2006) The genus lupines usually narrow down 36-52% protein, oil about 5-20% in addition to 30-40% of fiber (Mila; P. et al 2004) Nearby evaluations of lentil seeds indicated that the concentration of proteins and carbohydrates were 20.67g/100g and 60.47 g/100g, respectively. Flour contents of moisture, ash and lipids were 9.57, 0.97 and 3.22 g/100 g sample respectively. Carbohydrates represent are main element of lentils, which contain solvable and unsolvable dietetic fiber such as healthy prebiotic oligosaccharides (VidalValverde et al., 2002). (Sujak.et al, 2006) founded that Potassium oxalate is reducing uricase enzyme which associated of renal failure and behaved for initiation of established deficiency. The oxonate-preserved rats can attend as expedient animal perfect nephropathy, and number of additional assessments related to uric acid. This pattern has been applied to evaluate medicines that have emotional impact of purines defecation, to regulate nutritional factors troubled on serum urea salts, otherwise appraise probable satisfying causes in confident complaints attendant per uric acid. Lupines have a well quality of lipids, dietary fiber, vitamins, and minerals. There are variations in contents of protein among varieties and cultivars as a importance of the appearances of the emergent environments and varieties of topsoil as (28% to 48%) (Rodríguez-Ambry et al., 2005 and Martínez-Villaluenga et al., 2009). Dietry fiber granules of lupine seeds were weighing about 40% in highly quantity than that in different seeds. Lupine seeds require in height- stabilizes essential amino acids and similarly have substantial amount of oil approximately 5–20% in whole seeds. The seed comprises of starch quantities and elevated amounts of soluble non-starch polysaccharides almost (5% to 12%) and (30–40%) respectively. (Hall et al., 2005) and (Uzun et al., 2007). Legumes, as lupines, beans, are significant beneficent for colonic health because of the resistant starches which to be fermented by colonic bacteria. the observed that lupine kernel fibres were suppression appetite and lessening cholesterol belongings, lower in insulin levels and blood glucose although the antioxidant occupation were unknown (Wang and Daun 2006) and (Hall et al., 2005) Husk and cotyledons of fenugreek seeds, indicated that the highest of saponin (4.63 g/100 g) and protein (43.8 g/100 g) content in endosperm while, husk had more amount to polyphenols. At 200 mg meditation the obtains result of antioxidant activity on husk, and blood glucose, although photochemistry and antioxidant activity were unknown (Wang and Daun 2006) and(Hall et al., 2005))Husk and cotyledons of fenugreek seeds, indicated that the highest of saponin (4.63 g/100 g) and protein (43.8 g/100 g) content in endosperm while, husk had more amount to polyphenols. fenugreek leafs or seeds are widely consumed , in many countries as enhance in food preparations proper to its strong flavour and aroma, also as an ingredient in accepted medicine. It is rich origins on calcium, irons, and carotenes (Madhava et al., 2011). The evolution rats feed on sweet lupine flour containing 41.7% protein and 0.025% lupine, was highly significant than that of the controls through

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standard haematological parameters and tests of liver function. The insufficiency of protein have revealed to the contrary properties to the activity of immune system, causing in a advanced dangerous of toxicities (Amarowicz et al., 2008). The obtained antioxidant results were attributed to their constituents. Lentil have high quality of phenols and non-phenol antioxidants like catchiness and procyanidins. Total antioxidant measurements for lentil seeds were reported to be highly significant than other indicators through means of antioxidant capacity of different legumes (Xu et al., 2008) and Martínez-Villaluenga et al., 2009)

Fenugreek had significant level of biologically saponins which are recognized to advancing functions of liver and kidney. It's involve rich in proteins, containing lysine and Ltryptophan amino acids, mucilaginous fibre plus special chemicals components for example, coumarone, nicotinic acid, saponine, Phytic acid, Scopolamine and Trigonelline, which are believed to description for various accepted beneficial effects, may well inhibit absorption of cholesterol and emergence to improve higher levels of blood sugar (Syeda et al., 2008) and (Abd El-Ghany et al., 2011). The inducing GST in the rat livers consumed gash lentils might be the incidence of acceptable quantities of phytic acid that enriching hepatic GST levels. Lupine seeds Extracts have antioxidant performance have complete phenolic acids and flavonoid (Khatiwada et al., 2006) and (Siger et al., 2012)

5. RECOMMENDATION

The requirement of legumes reserve in alternative animal proteins is necessary to increase the improvement technical of old plant-resultant nutrients with swelling sentience of different nutritional practices on human. Lupine, and lentil seeds alone or combination with fenugreek seeds are recommended for renal disease patient, to be recycled for the production of another products as supplement for plant protein particularly in categories that necessitate protein for growth. Many researches are needed for examine the consequence of various cocking procedures on the suitability and accessibility of legumes.

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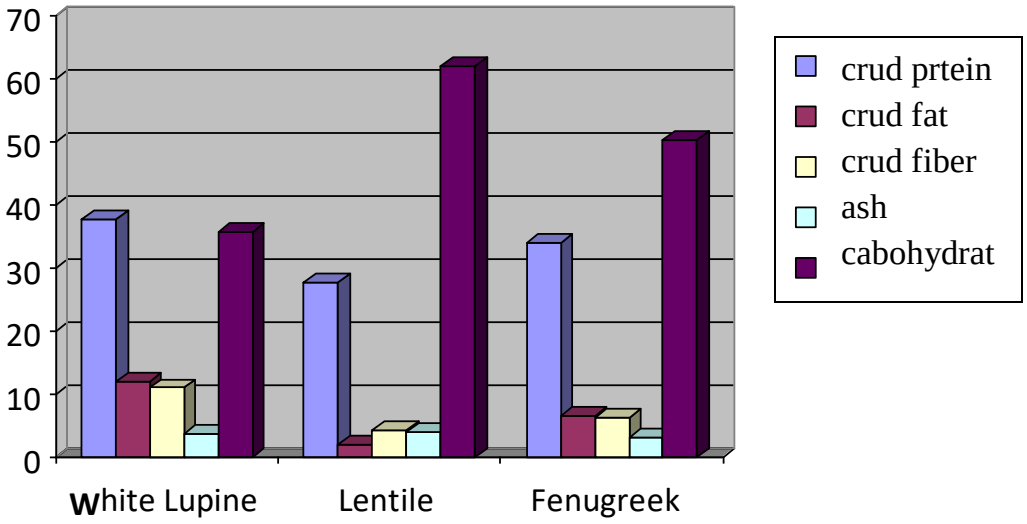
Table (1): Gross Chemical composition of lupines, lentil and fenugreek (100g/dry weight)

	Crude protein	Crude fat	Crude fiber	ash	carbohydrate
Whit Lupines	37.86	11.96	10.91	3.67	35.60
Lentil	27.81	1.86	4.22	4.01	62.10
Fenugreek	34.01	6.55	6.08	2.9	50.46



Gross Chemical composition of lupines, lentil and fenugreek (100g/dry weight)

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Table (2): Mean values ± SD of WG, WG %, FI ,FER,PI and PER of the experimental at groups

Groups Variables	Control -ve	Control +ve	Lupines	Lentils	Lupines with Fenugreek	Lentils with Fenugreek
WG(g)	89.41±3.14^{a}	48.77±2.11^{c***}	70.81±2.77^{b**}	72.70±3.25^{b**}	78.14±3.57^{b*}	83.21±3.14^{ab}
WG%	55.19±2.13^{a}	29.92±1.19^{c***}	43.17±2.15^{b**}	44.87±3.01^{b**}	47.93±2.18^{ab}	50.73±2.75^{a}
F I(g/d)	17.35±1.10^{a}	16.81±1.07^{a}	17.21±1.05^{a}	18.14±1.22^{a}	17.80±1.11^{a}	18.55±1.2^{a}
FER	0.053±0.001^{a}	0.029±0.003^{c***}	0.041±0.002^{b**}	0.042±0.007^{b**}	0.044±0.004^{ab}	0.045±0.005^{a}
P I (g/d)	3.47±0.31^{a}	3.36±0.30^{a}	3.44±0.2^{a}	3.62±0.21^{a}	3.56±0.42^{a}	3.71±0.33^{a}
PER	0.429±0.01^{a}	0.241±0.01^{d***}	0.343±0.03^{bc**}	0.334±0.04^{c**} [1]	0.365±0.05^{ab}	0.373±0.07^{ab}

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

Table (3): Mean values ± SD of uric acid, creatinine and urea of the experimental rat groups

Groups Variables	Control -ve	Control +ve	Lupines	Lentils	Lupines with Fenugreek	Lentils with Fenugreek
Uric acid (mg/dl)	c 2.55±0.34	a*** 8.64±1.14	b** 4.25±0.64	b** 4.17±0.73	bc 3.22±0.52	bc 3.19±0.29
Creatinine mg/dl)	d 0.70±0.04	a*** 1.77±0.22	b* 0.90±0.01	b* 0.91±0.02	c* 0.82±0.03	c* 0.80±0.02
Urea (µ /mg)	d 20.16±1.71	a* 53.71±4.22 **	31.015±3.18 b**	b 30.14±3.19 **	b* 28.81±2.75 *	bc 27.11±2.91 *

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

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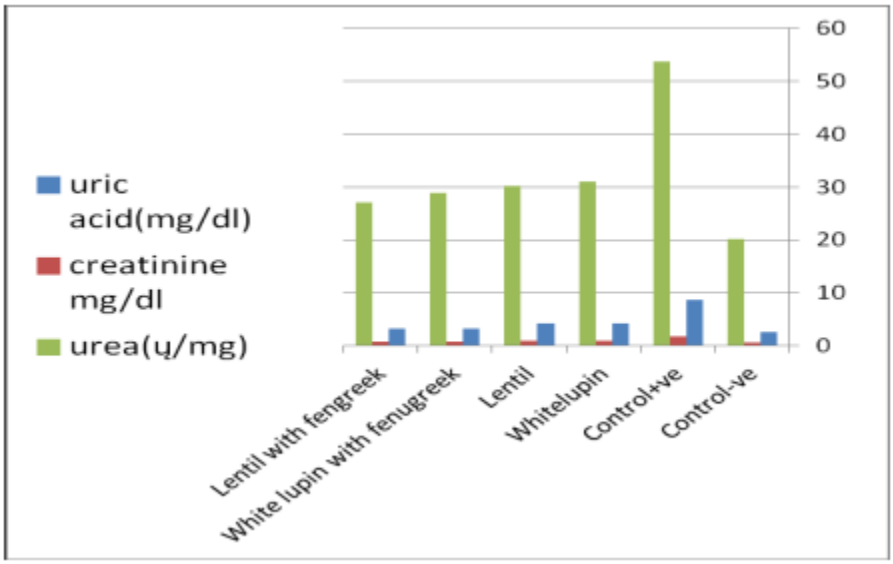

Table (4): Mean values ± SD of total protein, albumin and globulin of the experimental rat groups

Groups Variables	Control -ve	Control +ve	Lupines	Lentils	Lupineswith fenugreek	Lentilswith fenugreek
Total rotein(g/dl)	7.14±0.51^{ab}	6.11±0.62^{c**}	7.66±0.72^{a}	7.51±0.63^{a}	7.87±0.59^{a}	7.68±0.68^{a}
Albumin(g/dl)	3.35±0.41^{a}	3.50±0.30^{a}	3.61±0.42^{a}	3.54±0.41^{a}	3.70±0.31^{a}	3.66±0.22^{a}
Globulin (g/dl)	3.79±0.23^{a}	2.61±0.08^{b**}	4.05±0.30^{a}	3.97±0.27^{a}	4.17±0.25^{a}	4.02±0.11^{a}

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

Table (5): Mean values ±SD of serum SOD, catalase, GST, GPX, and NO, of the experimental rat groups

Groups Variables	Control -ve	Control +ve	Lupines	Lentils	Lupines with Fenugreek	Lentils with Fenugreek
SOD(µ/mg)	^{ab} 3.17±0.33	^{c***} 1.57±0.25	^{ab} 3.19±0.39	^{ab} 3.24±0.39	^{a} 4.21±0.48	^{a} 4.16±0.43
Catalase(µ /l)	^{a} 5.67±1.02	^{c***} 1.25±0.13	^{ab} 4.43±0.41	^{ab} 4.40±0.45	^{a} 4.59±0.50	^{a} 4.96±0.42

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GST(µ/mg)	a 4.21±0.59	c*** 1.87±0.29	b* 3.05±0.73	b* 3.11±0.45	a 3.77±0.43	a 3.82±0.62
GPX(µ/mg)	a 2.14±0.24	b*** 0.65±0.03	a 2.14±0.26	a 2.01±0.27	a 2.22±0.30	a 2.11±0.22
NO(µmOl/l)	bc 2.49±0.44	a*** 7.86±1.03	b 3.82±0.72	b 3.74±0.63	b 3.61±0.77	b 3.51±0.84

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

Table (6): Mean values ± SD of HG, PCV, Ig M and Ig G.of the experimental rat groups

Groups Variables	Control -ve	Control +ve	White Lupines	Lentils	White Lupines with fenugreek	Lentils with fenugreek
HG	12.77±1.11^{a}	10.67±1.21^{b**}	11.25±1.20^{a}	11.41±1.40^{a}	11.21±1.31^{a}	1.33±1.29^{a}
PCV	38.66±3.44^{a}	29.65±2.19^{c***}	32.96±3.14^{b} ^{c*}	33.66±3.29^{a} ^{b}	34.71±3.22^{ab}	35.61±3.11^{a}
Ig M (mg/l)	101.36±8.66^{a}	65.17±6.19^{c***}	85.61±8.19^{b} *	89.14±8.60^{b} *	98.11±9.41^{a}	99.66±8.67^{a}
Ig G (mg/l)	202.77±23.61^{a}	129.66±10.21^{e*} **	155.61±12.13c^{d**}	163.61±14.03^{c*}	190.21±15.19^{a}	185.14±14.19^{ab}

Significant with control (-ve) group * P 0.05 ** P 0.01 *** P 0.001

Values with the same letters in raw indicate non- significant difference (P 0.05) and vice versa