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The Effect of Some Animal and Plant Proteins on uric acid index in Rats with Acute Renal Failure

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Abstract

Kidney diseases are a public health problem all over the world. From recent studies it has been found that a low-protein diet as part of diet therapy has beneficial effects that slow the progression of chronic kidney disease. So, this study was carried out to investigate the uric acid index of some animal protein (Beef, eggs, kidney) and plant protein (mushroom, yellow lentils, lupine) in rats with induced-acute renal failure (ARF). Forty rats were divided into 8 groups (n=5) in each group. The first group of rats was fed on basal diet. The other rats were injected with one dose of 50% glycerol (10 ml/kg B.Wt.) in their hind limbs to induce ARF, these rats were divided into 7 subgroups, as follows: Subgroup (1): Rats with ARF were fed on basal diet supplemented with 150 gm/kg casein as positive control group (+Ve). From subgroups (2 : 7) rats were fed on the basal diet supplemented with 150 gm/kg from dried beef, eggs, kidney, mushroom, yellow lentils, lupine, respectively for 4 weeks. The treated groups with either animal or plant proteins had a significant decrease ($P<0.05$) in the level of kidney functions as well as lowering the mean values of phosphorous, sodium and potassium. The level of serum albumin and total protein were significantly ($P<0.05$) increased as compared to the +ve control group. It could be concluded that a diet containing animal protein (beef, eggs, kidney) or plant protein (mushroom, yellow lentils, lupine) may be used as a part of diet therapy to slow the progression of kidney disease and improve the kidney functions.

Key words: *Uric acid Index, Beef powder, Eggs, Kidney, mushroom, yellow lentils and lupine, Acute Renal Failure, Rats*

1. Introduction

Kidneys filter the blood to remove waste, nitrogen and retain water. They also control the ion concentrations and acid-base balance of the blood, metabolism of lipid, secretion of hormones and the production and utilization of systemic glucose. Kidney diseases are a public health problem all over the world (Crews et al., 2019). ARF is defined as the rapid decline in kidney include lowering glomerular filtration rate (GFR). The incidence of ARF increases with age and caused about 2 million deaths annually worldwide (Uchino et al., 2005; Ali et al., 2007 and Murugan and Kellum, 2011). ARF can cause end-stage renal disease directly and increase the risk of developing incident chronic kidney disease (CKD) (Chawla and Kimmel, 2012).

Creatinine is a chemical waste product produced by muscle metabolism. Kidneys filter creatinine and other waste products out of your blood. These waste products are removed from your body through urination (Mahan and Raymond, 2016).

A very low protein diet has beneficial effects in slowing the progression of CKD (Di Miccol et al., 2019). There is an association between different dietary protein sources and the risk of incident CKD. Red and processed meat were adversely associated with CKD risk, while nuts, low-fat dairy products, and legumes were protective against the development of CKD (Haring et al., 2017).

MATERIALS AND METHODS

Materials: Animal Protein (Beef meat, Eggs, Kidneys) and plant protein (Mushroom, Yellow lentils, Lupine) were obtained from the Egyptian local market

Chemicals: Glycerol 50%, casein, all vitamins, minerals, cellulose, choline and starch were obtained from El-Gomhoriya Company, Cairo, Egypt. Kits for Biochemical analysis: Kits required for estimating parameters used in the study were purchased from the Gamma Trade Company for pharmaceutical and chemicals, Dokki, Egypt. Animals: Adult male Sprague Dawley strain rats (n=40) which weighing about 200-205 g b. wt. were obtained from the Laboratory Animal Colony, Helwan, Egypt

Methods:

1) Induction of acute renal failure: Rats were given intramuscular injections of 50% glycerol (10 ml/kg B. Wt.) in their hind limbs (Midhun et al., 2012). Random blood samples are taken to analysis kidney functions to insure the incidence of ARF.

2) Preparation of protein powder: The plant and animal protein were dried at the Research Center Solar power Unit and laid after being crushed and sifted.

3) Chemical Determination: Chemical determination of moisture, protein, and fats were determined according to A.O.A.C., (2005) and carried out at Graduate laboratory, Faculty of Home Economics, Helwan University.

4) Preparation of basal diet: The basal diet (AIN-93M) was prepared according to Reeves et al., (1993). It consists of 14% (casein<85%), **4**% corn oil, 0.2 % choline chloride, 1% vitamin mixture, 3.5 % salt mixture, 5% cellulose, 10% sucrose and the remainder was corn starch (The quantity of casein was replaced with the same quantity of the tested protein).

Experimental design: Rats were housed in well conditions in Biological Studies Lab of Faculty of Home Economics, Helwan University. They were kept in standard cages at room temperature (25 ± 3°C) with a 12 h dark/light cycle. They were left for seven days as adaptation period, and they were allowed to feed standard laboratory food and water. Forty rats were divided into 8 groups (n=5) in each group. The first group of rats was fed on basal diet. The other rats were injected with one dose of 50% glycerol (10 ml/kg B.Wt.) in their hind limbs to induce AFR. These rats were divided into 7 subgroups, as follows: Subgroup (1): Rats with ARF were fed on basal diet supplemented with 150 gm/kg casein as positive control group (+Ve). Subgroup from (2 : 7) rats were fed on basal diet supplemented with 150 gm/kg from dried beef, eggs, kidney, mushroom, yellow lentils, lupine, respectively.

At the end of experiment (4 weeks), rats were anesthetized; Blood samples were collected from the aortic vein into clean dry centrifuge tubes and stored at room temperature for 15 minutes, put into a refrigerator for 2 hour, then centrifuged for 15 minutes at 3000 rpm to separate serum. Serum was carefully aspirated and transferred into dry clean Wassermann tubes by using a Pasteur pipette.

Biological evaluation: was carried out by determination of feed intake (FI) throughout the experimental period, body weight gain % (BWG %) and feed efficiency ratio (FER) were determined according to Chapman et al., (1959) using the following equations:

BWG % = (final body weight – Initial body weight / Initial body weight) × 100

FER = Weight gain (g)/ Feed intake (g).

Biochemical Analysis: Serum was used to determine Kidney functions as (uric acid, creatinine and urea) according to methods described by Young, (2001). Sodium (Na), Potassium (K) were measured according to the colorimetric method of Henry, (1974) and Henry, (1964), respectively. Phosphorus (PO4) was estimated according to methods described by El-Merzabani et al., (1977). Serum total protein and albumin were estimated according to methods described by Weissman et al., (1950).

Statistical analysis: All data obtained results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). The collected data were presented as mean± standard error (SE). Analysis of Variance (ANOVA) test was used for determining the significances among different groups. All differences were considered significant if P-values were ($P < 0.05$) (Armitage and Berry, 1987).

RESULTS AND DISCUSSION:

The data in Table (1) shows the effect of Some Animal and Plant Proteins on Creatinine Index in Rat with Acute Renal Failure on body weight status.

Table 1: Effect of Some Animal and Plant Proteins on body weight status in Rats with ARF.

Parameters / Groups	IBW (g)	FBW (g)	BWG%	FI(g/day/ rat)	FER
Control (-ve)	204.60±0.94a	329.06±1.72b	60.84±1.10b	29.00	0.095±0.01de
Control (+ve)	203.65±0.94a	338.45±3.07a	66.22±2.15a	30.00	0.099±0.02cd
Yellow lentils	205.00±0.70a	317.45±1.99c	54.86±1.32c	24.50	0.102±0.03bcd
Lupine	205.45±0.85a	317.60±1.46c	54.60±1.05c	27.00	0.092±0.01e
Mushroom	205.15±1.36a	315.72±1.85c	53.93±1.69c	23.00	0.106±0.03abc
Eggs	204.55±1.07a	320.92±2.07c	56.90±1.03bc	23.00	0.110±0.01a
Beef meat	203.50±1.11a	314.40±2.15c	54.51±1.27c	23.34	0.105±0.02abc
Kidneys	205.00±0.70a	316.12±1.73c	54.22±1.24c	22.70	0.108±0.02ab

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at (P<0.05).

According to the initial body weight there is no significant difference among all groups. While the data demonstrating the FBW showed that there is a significant increase (P<0.05) in the FBW for the +ve control group as compared to the negative control group. The supplementation with either animal or plant proteins significantly (P<0.05) decreased the FBW, BWG% while FER are significantly increased as compared to the +ve control group. It was clear also that there are no significant differences in the FBW and BWG% among the treated groups. There is a numerically lowered FI among the treated groups as compared to the +ve control group. There are no statistical changes in FER among the groups treated with dried egg, beef, kidneys, and mushroom.

Protein is critical for the development of bone and muscle mass. However, extra high protein intake can result in side-effects due to imbalance in energy intake and food consumption (Lin et al., 2015). Dietary fibers play an important role in body weight regulation, through both hunger suppression and diminished nutrient absorption (Henness and Perry 2006). Plant proteins are high in fiber, low in energy density and glycemic load, and moderate in protein are thought to be particularly important for weight control (Albete et al., 2010 and Luis et al., 2017). Lin et al., (2015) indicate that plant protein had a more protective effect against obesity compared to animal protein.

Evidence shows that plant protein from vegetables, fruits, and legumes not only improves body composition, but also results in lower body weight compared to animal protein (Hermanussen, 2008**.** Therefore, the amount of total, animal and plant proteins in the diet may be a critical factor on prevention against obesity and overweight. Evidence also shows that increasing protein intake results in improvement of serum lipids (Rolland et al., 2009).

Consistent with our findings, observed benefits of increasing total and plant protein intakes on body composition (Bradlee et al., 2010 and Van Vught et al., 2009) could be attributed to the protein effect on increasing stimulated fat oxidation and building of lean body mass (Soenen et al., 2010).

Al-Amoudi, (2013) suggested that rats of renal failure without dialysis and fed on fish and white bean showed the highest significant increase in BWG when compared to control positive group, while groups fed on meat, chicken and egg showed the lowest significant decrease in BWG when compared to control positive group **.**

Table (2): Effect of Some Animal and Plant Proteins on serum Albumin and Total protein in rat with ARF.

Parameters / Groups	Albumin	Total protein
	(g/dl)	
Control (-ve)	7.12±0.25ab	10.10±0.40a
Control (+ve)	3.50±0.26f	4.72±0.21e

Yellow lentils	5.75±0.15cd	6.60±0.28d
Lupine	5.37±0.17de	7.27±0.23d
Mushroom	6.03±0.15c	7.45±0.31d
Eggs	7.37±0.20a	9.20±0.21b
Beef meat	6.72±0.15b	8.30±0.29c
Kidneys	4.82±0.12e	6.77±0.20d

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

As shown in table (2) of biochemical parameters on ARF animal model included serum albumin was significantly lowered (P<0.05) for the +ve control rats when compared to the -ve control group. While there are no significant changes in serum albumin for the groups treated with Egg or beef meat when compared to that means of the -ve control group. All tested animal or plant protein significantly (P<0.05) increased the mean value of albumin as compared to the +ve control group. Mushroom caused the highest increase in serum albumin among the plant proteins, while eggs followed by beef meat caused the highest increase in albumin value among the plant and animal proteins.

Regarding to total protein value, ARF rats had significant decrease of total protein when compared to the -ve control group. On the other hand, the tested materials caused a significant increase (P<0.05) in the total protein value as compared to the +ve control group. It was obvious that there are no changes in serum total protein among the plant proteins groups. While there are significant differences among the tested animal proteins. Eggs supplementation caused the highest increase in serum total protein among the other treated rats.

Low albumin levels can be a sign of liver or kidney disease or another medical condition (McPherson and Pincus, 2017). Konda et al., (2016) indicated a significant decrease in albumin and total protein level after the induction with glycerol as compared to the negative control group confirming renal failure., these results agreed with the current study.

The obtained results are agreed also with the results of Yanpallewar et al., (2003) and Al-Amoudi, (2013), found that serum Albumin and T. Protein levels in treated groups with meat and chicken were significantly increased.

According to the Protein Digestibility Corrected Amino Acid Score (PDCAAS), a measure of a protein's ability to provide adequate levels of essential amino acids, whole egg scores a "1" on a scale of 0 to 1. As measurement of dietary protein utilization, the biological value of eggs (100) is exceeded only by whey protein (104) (Hoffman et al., 2004). Egg intake has been demonstrated to have an inverse relationship with dialysis vintage, which may be attributed to loss of interest in food or cooking. Eggs are a rich source of leucine, an essential amino acid that plays an important role in muscle protein synthesis. Both increased muscle proteolysis and impaired muscle protein synthesis are commonly associated with CKD (Wang et al., 2014).

Therefore, oral protein supplementation (OPS) has been used as a solution to overcome dietary protein inadequacy. To date, only one OPS study using egg albumin as a renal-specific product has been conducted (Jeloka et al., 2013).

Table (3): Effect of Some Animal and Plant Proteins on PO4, Na and K in rat with ARF

Parameters / Groups	PO4	Na	K
	(Mmol/L)		
Control (-ve)	4.77±0.29d	112.55±0.68f	3.12±0.26d
Control (+ve)	8.67±0.12a	164.50±1.85a	5.70±0.13a
Yellow lentils	6.82±0.10b	138.57±2.72c	5.02±0.11b
Lupine	6.77±0.12b	144.89±1.24b	4.72±0.20b
Mushroom	6.55±0.21b	140.94±2.01bc	5.00±0.10b
Egg	6.02±0.17b	125.42±1.23e	3.65±0.19cd
Beef meat	5.85±0.27c	131.72±2.91d	3.95±0.26c
Kidneys	6.80±0.16b	138.10±1.21c	4.05±0.27c

Values are expressed as means ± SE.

Values at the same column with different letters are significantly different at P<0.05.

Data in table (3) revealed that serum Na, PO4 and K were significantly (P< 0.05) increased in the +ve control group compared with the -ve control group. Results showed that all treated groups with Plant Protein or animal proteins had significant decrease (P<0.05) in serum Na, PO4 and K as compared to the +ve control group. Regarding the value of phosphorus, there are non-significant differences among the rats treated with different plant proteins. Also, there are no changes in serum PO4 between the groups treated with egg or kidneys. It was clear that yellow lentils caused a significant decrease in Na value as compared to lupine supplementation. On the other hand, there is a significant difference among the groups treated with the animal proteins. Moreover, there is no significant change in the level of K among the treated groups with plant proteins, the same trend was observed among the rats treated with the animal proteins.

Potassium measurements are used to monitor electrolyte balance in the diagnosis and treatment of hypo/hyperkalemia, and diseases involving electrolyte imbalance. Hypokalemia associated with low total body potassium is either due to poor dietary intake or increased potassium loss from the body (Appel et al., 2006) Hypophosphatemia is usually secondary to the inability of the kidney to excrete phosphate as it occurs in renal failure (Azevedo et al., 2003). The result of serum K+, Na and PO4 in Table (3) agreed with Uchendu et al., (2017), who reported that the level of K+, Na and P were increased significantly, in the group injected with glycerol as compared with normal group , this was as a result of the damage to the proximal convoluted tubule (Gekoski et al.,2012). Gilbert and Weiner, (2022), reported the PO4 and K from beans are not absorbed as well as the phosphorus and potassium from animal sources or phosphate and potassium additives. Also, the result of serum sodium agreement with Guenther et al., (2006) and Brick & Thompson, (2006) who reported that beans are very low in sodium. Foods rich in these nutrients, except for sodium, are fruits, vegetables, whole grains, beans and legumes, which aid in reducing production of inflammation markers and oxidative stress (Sahathevan et al., 2018).

Table (4): Effect of some animal and plant proteins on kidney functions of rat with ARF

Parameters / Groups	Uric acid	Creatinine	Urea
	(mg/dl)		
Control (-ve)	1.50±0.14f	0.46±0.05c	25.61±1.04d
Control (+ve)	5.97±0.21a	1.60±0.17a	62.50±2.29a
Yellow lentils	3.86±0.13b	0.83±0.04b	42.00±1.76b
Lupine	3.06±0.08c	0.92±0.02b	43.72±2.39b
Mushroom	3.87±0.21b	0.84±0.04b	40.67±2.22bc
Eggs	1.86±0.13ef	0.69±0.03b	29.35±1.53d
Beef meat	2.26±0.27de	0.76±0.03b	35.45±1.34c
Kidneys	2.68±0.26cd	0.80±0.06b	38.00±1.83bc

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.
As seen in table (4) The biochemical analysis of uric acid showed that there is a significant elevation of serum uric acid, creatinine and urea level in the ARF rats' group as compared to the -ve control group. Treatment with different tested plant or animal proteins caused a significant (P<0.05) decrease in serum uric acid, creatinine and urea level when compared to the +ve control rats. It was demonstrated that there are no significant changes in serum creatinine among all the treated rats. Regarding the uric acid value, there is no change between the groups treated with either yellow lentils or mushroom, and between eggs or beef meat and between beef meat or kidneys. There are no significant differences in serum urea among the plant proteins (yellow lentils, lupine, mushroom). Moreover, no significant changes were observed between the groups treated with either beef meat or kidneys. The lowest reduction in serum kidney functions is recorded at the group treated with eggs.
Higher plant protein and lower animal protein intake lead to consumption of higher proportions of glutamic acid, proline, phenylalanine, cysteine and serine. This difference in amino acids could be the reason for the different effects of plant and animal protein on kidney function (Elliott et al., 2006). Plant

protein may be helpful to lessen oxidized-lipoprotein–induced glomerular damage and progression of CKD by reducing serum lipids levels (Tovar et al., 2002). Plant-based sources of proteins are also rich in calcium, magnesium, potassium, and vitamin C, which were associated with lower dietary acid load and improvement in kidney function (Mirmiran et al., 2016). These explanations can support the findings of the current study.

Patients with chronic kidney failure are advised to consume a diet with modest protein restriction in order to limit the development of toxic nitrogenous metabolites, uremic symptoms and other metabolic complications (Group, 2009). However, information is lacking in regard to whether different dietary proteins may have dissimilar impact on kidney function, and it is of interest that intake of fish has been associated with reduced risk of developing kidney disease (Gopinath, et al., 2011).

Yuzbashian et al., (2014) revealed that a higher intake of plant protein was significantly associated with a lower risk of prevalent chronic kidney disease. Alvirdizadeh et al., (2020) confirmed an inverse association between plant protein intake and the risk of incident CKD, which demonstrates the protective role of plant-based protein in a diet on kidney function.
Finally, it could be concluded that a diet containing animal Protein (Beef meat, Eggs, kidneys) and plant protein (mushroom, yellow lentils, lupine) may be used as a part of diet therapy to slow the progression of kidney disease.

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