THE EFFECT OF SOME ANIMAL AND PLANT PROTEINS ON CREATININE INDEX IN RAT WITH ACUTE RENAL FAILURE

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Abstract

Kidney diseases are 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 creatinine index of some animal protein (Camel meat, rabbit meat, Tilapia) and plant protein (Popcorn, brown lentils, chickpeas) in rats with induced-acute renal failure (ARF). Forty rats weighing approximately 170-180 g, were divided into 8 groups (n=5) in each group. The first group of rats (normal) was fed on basal diet. The ARF rats were divided into 7 groups, one of which was a positive control group and fed on basal diet until the end of the experiment. The rest of the groups were fed on basal diet fortified with 150 g of dry powder meat of camel, rabbit, tilapia, popcorn, brown lentils and chickpeas, respectively. The treated groups with either animal or plant proteins had a significant decrease ($P<0.05$) in the level of kidney functions parameters as well as lowering the mean values of PO_4, Na and K. The level of serum albumin and total protein were significantly ($P<0.05$) increased as compared to the positive control group. It could be concluded that a diet containing animal protein (Camel meat, rabbit meat, Tilapia) and plant protein (popcorn, brown lentils, chickpeas) may be used as a part of diet therapy to slow the progression of kidney disease. These diets may be beneficial for patients who suffer from acute renal failure.

Key words: Creatinine Index, Camel meat powder, rabbit meat, Tilapia, Popcorn, brown lentils and chickpeas, Acute Renal Failure, Rats

INTRODUCTION

Kidneys filter the blood in order to make urine, to release and retain water and to remove waste and nitrogen. They also control the ion concentrations and acid-base balance of the blood, metabolism of lipid, secretion and degradation of hormones and the production and utilization of systemic glucose. Each kidney feeds urine into the bladder by means of a tube known as the ureter (**Cotran *et al*., 2005**).

Kidney diseases are public health problem all over the world (**Crews *et al*., 2019**). ARF is defined as the rapid decline in kidney function as manifested by a reduction in glomerular filtration rate (GFR). It is a more frequent problem observed in all hospital admission. The incidence of ARF increases with age, and is responsible for approximately 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 (ESRD) directly, and increase the risk of developing incident chronic kidney disease (CKD) and worsening of underlying CKD (**Chawla and Kimmel, 2012).**

A very low protein diet as a part of nutritional therapy have beneficial effects in slowing the progression of CKD (**Di Miccol *et al.*, 2019)**. Another study reported associations of different dietary protein sources with the risk of incident CKD and reported that 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)**.

Creatinine is a chemical waste product produced by muscle metabolism. When your kidneys are functioning normally, they filter creatinine and other waste products out of your blood. These waste products are removed from your body through urination. It is involved in the supply of energy for muscular contraction **Mahan and Raymond, 2016)**.

MATERIALS AND METHODS

Materials: Animal proteins (camel meat, rabbit meat, tilapia) and plant proteins (corn, brown lentils, and chickpeas) 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 170-180 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**).

2) Preparation of protein powder: The plant and animal protein were dried and laid after boiled first, being crushed and sifted then stored.

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).

5) Experimental design: Animals were housed in well conditions in biological studies lab of Faculty of Home Economics. Rats were kept in standard cages at room temperature (25-28°C) with a 12 h dark/light cycle. It was left for seven days as adaptation period and was allowed to feed standard laboratory basal diet and water.

The present study was done on (40 rats) weighing approximately 150-180 g,

Animals were divided into 8 groups (5 rats each). The first group of rats (normal) was fed on basal diet. The ARF Rats were divided into 7 groups as follows: Group (2) was fed on basal diet containing 150 g Casen /kg diet and kept as (positive control). Groups (3, 4, 5) were fed on basal diet containing 150 g/kg diet of dry powder of Camel meats, rabbit meats, Tilapia, respectively. Groups (6,7,8) were fed on basal diet containing 150 g/kg diet of dry powder of Popcorn, brown lentils, chickpeas, respectively.

At the end of experiment (30 days), rats were anesthetized; blood samples were collected from hepatic portal vein in clean centrifuge tubes and serum was separated.

Biological evaluation: was carry 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 % = (Initial body weight – final 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) was measured according to the colorimetric method of **Henry, (1974**). Potassium (K) was estimated according to the colorimetric method of **Henry, (1964**). Phosphorus (PO_4) 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: The results were expressed as mean ± standard error (SE). The statistical analysis was carried out by using SPSS, PC statistical software (Version 18.0 SPSS Inc., Chicago, USA). Data were analyzed by one way analysis variance (ANOVA). The values were considered significantly different at $P < 0.05$ **(Snedecor and Cochran, 1980).**

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 initial body wight, final body wight, body wight gain presents, feed intake and food efficiency ratio are shown in Table (1).

Table 1: Effect of Some Animal and Plant Proteins in Rat with Acute Renal Failure on body weight status of rats with induced ARF.

Groups \ Parameters			IBW (g)	FBW (g)	BWG%	FER	FI(g/d/rat)
control		**(-ve)**	205.25±1.93^{a}	296.70±1.78f	44.63±1.91f	0.078±0.027d	23.00
		(+ve)	204.20±0.85^{a}	342.70±1.13a	67.84±0.93a	0.099±0.010a	31.00
Treatments	Plant	**Corn**	203.87±1.41^{a}	327.35±2.23b	60.60±1.64b	0.093±0.020b	29.50
		Brown lentils	205.62±1.19^{a}	320.85±1.79c	56.06±1.26c	0.089±0.017bc	28.50
		Chickpeas	205.22±2.83^{a}	316.35±1.35d	54.29±2.20cd	0.088±0.024bc	28.00
	Animal	**Camel meat**	204.40±0.78^{a}	309.19±1.20e	51.28±1.11de	0.087±0.016bc	26.50
		Rabbit	205.32±1.68^{a}	305.77±0.84e	48.98±1.48e	0.085±0.019c	26.00
		Tilapia Fish	204.45±0.68^{a}	305.25±1.13e	49.30±0.59e	0.087±0.009bc	25.60

Results are expressed as mean ± SE.

Values in each column which have different letters are significantly different at ($P<0.05$).

As seen in table (1) the IBW was recorded before started the experiment, so that it was no significant different in the initial body weight of all the experimental groups. The mean values of the FBW, BWG % and FER for the +ve control group were significantly increased ($P<0.05$) as compared to the -ve control group. Supplementation with either plant or animal proteins caused a significant decrease in the mean values of FBW, BWG % and FER as compared to the -ve control group. Furthermore, there were no significant differences in the mean of FBW, BWG % and FER among the groups treated with animal proteins. The highest improvement in FBW, BWG %

and FER was observed at the group fed on basal diet supplemented with corn protein as compared to the other treated groups.

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)**. This result was within opinion whom reported that chickpea 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 more protective effect against obesity compared to animal protein. **Wei *et al.*, (2022)** indicated that Corn could effectively alleviate weight gain in rats. **Nagib, (2022)** indicated that, treating rats with chickpea led to significant decrease in body weight gain. plant-protein sources contain higher amounts of fiber that has been found to be related to improved metabolic status **(Chen et al., 2018).**

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)**. **Lin *et al.*, (2015)** concluded that total and animal protein intakes might be responsible for increasing body weight and BMI in adolescents. A randomized 8-weeks parallel intervention trial suggested that seafood protein sources from cod and salmon were efficient to treat obesity because of caloric restriction and lower saturated fatty acids intake **(Parra *et al.*, 2007)**. 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)**.

In addition, **Refaat *et al.*, (2022)** showed significant decrease (P 0.05) in body weight gain and FER of control positive (suffering from osteoporosis (compared to control negative (healthy rat).These results are accordance with the results obtained by **Gouda (2012)**, who reported that control positive (osteoporotic rats) showed significant decrease in body weight gain .**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 .

These results are agreed with **EL- Sayed, (2017)** showed that rats which were fed on basal diet containing fish as source of protein recorded higher feed intake and BWG value than other treated groups. Feed intake and BWG was increase in the negative control group than that of positive control group. On the other hand, all treatments illustrated significant decrease when compared them with negative control group. **Daidj and Lamri-Senhadji, (2021)** observed that fatty fish such as sardines help the body burn fat in addition to generating a feeling of satiety. This satietogenic effect may explain the decrease in food intake in obese groups consuming sardine oil and explains the negative FER values.

Al-Amoudi, (2013) suggest that rats with renal failure without dialysis and fed on fish and chicken showed the highest significant decrease in FI when compared to the positive control group, and at the same time, these groups revealed no significant changes when compared to normal rats . **Al-Amoudi, (2013)** suggest that showed that there was significant decrease in the feed efficiency ratio in the positive control group as compared to the negative control group. In rats with renal failure without dialysis, which was fed on fish and white bean showed the highest significant increase in FER when compared to the positive control group. These results confirm by the findings, which demonstrate that even patients with a mild decline in GFR (i.e., <50 mL/min) show decreases in their calorie and protein intake as dietary protein intake progressively declines with decreasing GFR **(Mehrotra, and Kopple, 2001)**. Similarly, a study reported that there is a limited decrease in food intake from 7.26 to 7.15 g/day (not significant) in normal rats and from 18.61 to 14.72 g/day (significant) in rats with renal impairment **(El-Moselhy, 2006).**

As shown in table (2) the mean values of serum albumin and total protein concentrations for the positive group was significantly (P<0.05) decreased as compared to the negative control group. The concentrations of serum albumin and total protein were significantly (P<0.05) increased for all the treated groups as compared to the positive control group. There were no significant differences in the concentrations of serum albumin and total protein between the groups treated with plant proteins (corn and brown lentils). The same trend was observed between the groups treated with brown lentils and chickpeas. In addition, there was no changes in serum albumin and total protein between groups fed on rabbit or tilapia fish. The highest significant (P<0.05) levels of serum albumin and total protein were observed for the groups fed on animal proteins especially rabbit or tilapia fish protein.

Table (2): Effect of Some Animal and Plant Proteins on Albumin and Total protein in rat with acute renal failure

Groups \ Parameters			Albumin	Total protein
Control		(-ve)	7.12±0.33b	10.10±0.52a
		(+ve)	3.50±0.34e	4.72±0.27f
Treatments	Plant	Corn	5.03±0.32d	6.25±0.25e
		Brown lentils	5.30±0.15cd	6.90±0.10de
		Chickpeas	5.93±0.10c	7.24±0.31d
	Animal	Camel meat	7.09±0.20b	8.50±0.44c
		Rabbit	7.88±0.13a	9.08±0.22bc
		Tilapia Fish	7.99±0.15a	9.56±0.12ab

Values are expressed as means ± SE.

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

Nagib, (2022) indicated that, treating groups with HFD containing chickpea improved the parameters of liver enzymes, as compared to the group which treated with HFD only. **Mekky et al., (2016)** showed that the chickpea extract showed a strong hepatoprotective activity based on assessing levels of albumen, total protein. The oral administration of chickpea extract exerted significant hepatoprotective activity by reduction of the aforementioned parameters compared to the reference drug silymarin this complies with results described previously **(Santhoshi *et al.,* 2013 and Sri Ramachandra et al., 2014)**. This activity may be attributed to the characterized phenolics **(Kinjo *et al.,* 2006)**.

Al-Amoudi, (2013) showed that all rats of renal failure without dialysis and fed on all tested protein sources reveal a significant increase in total protein when compared to control positive group. in addition all groups show significant increase in serum albumin when compared to control positive group. These results are agreed with **Negm, (2018)** and **Refaat *et al.*, (2022)** revealed that three type of fish caused a significant decrease in serum liver enzymes and parathyroid hormone. **Saka *et a*l., (2011)** showed that elevation of transaminase activities, in (AST), is linked to liver damage. Moreover, experimental studies have shown beneficial effects of diets rich of PUFAs on (AST) activities **(Ketsa and Marchenko, 2014)**. **Soltan, (2013)** and **EL- Sayed, (2017)** found that supplementation with fish meat which were added to the diet of osteoporotic rats led to decrease the mean value of liver enzymes significantly were noticed in comparison to control positive group.

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

Parameters / Groups	PO_4	Na	K
Control (-ve)	4.77±0.38d	112.55±0.88f	3.12±0.34d
Control (+ve)	8.67±0.16a	164.50±2.39a	6.42±0.21a
Corn	6.64±0.23b	141.25±2.50bc	5.25±0.29b
Brown lentils	6.93±0.23b	146.27±2.03b	5.10±0.16b
Chickpeas	6.46±0.27b	139.72±2.46c	5.35±0.22b
Camel meat	6.51±0.17b	127.72±1.01d	4.05±0.22c
Rabbit	5.67±0.31c	120.32±2.44e	3.40±0.40cd
Tilapia Fish	6.56±0.15b	118.30±1.48ef	3.10±0.33d

Values are expressed as means ± SE.

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

The mean values of serum PO_4, Na and K concentration in the +ve control group were significantly increased as compared to the -ve control group as shown in table (3). The treated groups with either animal or plant proteins had significant decrease in the level of PO_4, Na and K as compared to the +ve control group. There are no significant differences in the level of PO_4 and K among the groups treated with plant proteins. The lowest decrease in the mean of PO_4 was cleared at the rats treated with Rabbit, while Fish or Rabbit caused the lowest reduction for Na. on the other hand Camel milk or rabbit caused the lowest decrease in the level of K.

A study demonstrated that the fully defatted fish protein diet does not improve the renal failure compared with the control casein diet in spontaneously hypercholesterolemic rats, whereas a semi-defatted fish protein diet can improve renal function **(Liang *et al*., 2007)**.This suggests that the beneficial effect of the fish protein diet depends on the accompanying fish oil since the fish protein source includes 10% fat .

Al-Amoudi, (2013) suggest that all rats of renal failure without dialysis and fed on all tested protein sources revealed significant decrease in serum Na while showed significant increase when compared to control positive group. In the advanced stages of CKD, recommendations are to monitor potassium, phosphorus, sodium, and protein **(Marks *et al*., 2018)**. 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 acute renal failure

Parameters / Groups	Uric acid (mg/dl)	Creatinine	Urea
Control (-ve)	1.50±0.18e	0.45±0.06c	25.61±1.34d
Control (+ve)	5.97±0.27a	1.60±0.22a	62.50±2.95a
Corn	4.05±0.07b	0.97±0.08b	44.12±2.53b

Brown lentils	3.27±0.17c	0.88±0.03b	45.15±3.09b
Chickpeas	3.60±0.17bc	0.86±0.04b	40.67±2.87b
Camel meat	2.26±0.15d	0.59±0.02c	32.95±1.46c
Rabbit	1.95±0.20de	0.59±0.04c	30.75±0.86cd
Tilapia Fish	1.88±0.29de	0.55±0.03c	25.84±1.06d

Values are expressed as means ± SE.

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

The kidney functions of the rat with induced acute renal failure were significantly increased ($P<0.05$) as compared to the -ve control group (Table 4). On the other hand, rats treated with plant or animal proteins had significant decreased ($P<0.05$) in kidney functions as compared to the +ve control group. There were no significant changes in the level of creatinine or urea among the groups treated with plant proteins. The same trend was cleared for the animal protein for the same uric acid and creatinine concentrations. The highest improvement in kidney functions were observed at the rats treated with either Rabbit or Fish as compared to the other treated groups.

Several potential mechanisms can explain the association of dietary plant protein with kidney function. 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)**. It is reported that higher intakes of plant protein from gluten can reduce serum triacylglycerol, oxidized low-density lipoprotein (LDL) cholesterol and uric acid **(Jenkins *et al*., 2001)**. Plant protein may be helpful to lessen oxidized-lipoprotein–induced glomerular damage and progression of CKD by reducing these 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 (CKD). **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.

The previous results were in line with **EL-Sayed, (2017)** who the finest results of the mean values for serum uric acid, urea nitrogen and creatinine for the group which fed on diet containing fish as source of protein. **Drotningsvik *et al*., (2019)** showed that dietary consumption of salmon fish led to lower urine concentrations of several established renal function markers in Zucker fa/fa rats, indicating a better kidney function.

Al-Amoudi, (2013) showed that rats fed the fish protein diet had lower blood urea nitrogen (28.0 mg/dL), uric acid (3.5mg/dL) levels compared with the other groups. There were no significant differences in creatinine levels between rats fed the animal protein (fish and meat) or the plant protein (soy and white bean) diets .

Aycart *et al*., (2021) showed that the vegetarian diet significantly reduced CRP levels compared to the free and Mediterranean diets. The researchers attributed this to the low serum levels of urea that suppressed the rise in inflammation markers (**Di *et al*., 2018). Refaat *et al*., (2022)** revealed that three type of fish caused a significant decrease in serum urea, creatinine, and uric acid.

Finally, it could be concluded that a diet containing animal Protein (Camel meat, rabbit meat, Tilapia) and plant protein (Popcorn, brown lentils, chickpeas) may be used as a part of diet therapy to slow the progression of kidney disease.

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