EFFECTS OF HIGH PLANT COWPEAS (Vigna unguculata) AND ANIMAL (CASEIN) PROTEIN INTAKE ON KIDNEY MORPHOLOGY

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ABSTRACT

The relationship between high protein diet and morphological changes of the kidney in animal model has been established, but the emphasis has been on animal proteins. In developing countries like Nigeria, cowpea which is high in protein is one of the common sources of protein and because of its availability and cost it is highly consumed by the general populace. It is the aim of this study to determine the effect of the intake of plant (cowpeas) and animal proteins (casein) as high protein diets on renal morphology.

One hundred and seventy Wistar rats were used in this study; the rats were randomly distributed into 8 experimental groups (20 per group) and control (10). They were fed the experimental diets for six months at three months 10 from each group were sacrificed and the remaining 10 sacrificed at 6 months, the right kidneys were removed and put in formal saline for histological analysis.

Results show varied damages in renal tubular epithelium, glomeruli and arteries in rats fed with the casein diet. However, rats fed with the cowpeas constituted diets only showed mild morphological changes. This study has shown that, high casein diet, an animal protein, results in morphological changes in the kidney, while intake of cowpeas a plant protein only resulted in mild morphological changes.

Keywords: *High animal, plant, protein, kidney, morphology*

INTRODUCTION

In recent times, the use of high protein diet in weight control is gaining popularity. In the developed countries obsessed subjects are placed on high protein diets in order to lose weight. The DrDukan diet which is a hyper protein diet is one of such diets. Dietary reference guideline of protein for rats is 5% for maintenance; 15% for growth and lactation [1] while humans require 0.8g/kg body weight per day [2]. In developing countries like Nigeria, cowpea which is high in protein is one of the common sources of protein and because of its availability and cost it is highly consumed by the general populace. It is not uncommon to see diabetics consuming a lot of cowpeas in the form of porridge, moimoi and bean cake (Akara). Knowing that diabetics do come down with diabetic nephropathy in some cases, it is important for us to look at the effect of this highly consumed plant protein on the kidney.

The relationship between high protein diet and morphological changes of the kidney in animal model has been established but the emphasis has been on animal proteins. Some studies performed in rodents and pigs have shown histological damages with high protein diets in the long term intake[3,4,5]. It was reported that intake of high whole plant and animal proteins in proportions that mimicked human diets given to pigs resulted in enlarged kidneys at both 4 and 8 months[5], renal and glomerular volumes were 60–70% higher by the end of the study. These enlarged kidneys had greater evidence of histological damage, with 55% more fibrosis and 30% more glomerulosclerosis. Similarly, they also observed morphological impairment in the high protein diet fed group, with a 32% higher renal mesangium area, a 30% higher floccules areas and a 13% higher glomerular area in the high protein diet groups, but without significant more renal interstitial connective tissue. According to Aparicio *et al.*[6], one of the first histological lesions observed when the kidney is challenged is

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hyperplasia of the tubular epithelium which is presumed to be a compensatory phenomenon resulting from changes in the filtering ability of the glomerular tuft. From search in literature, not much work has been done on the effect of plant protein on the kidney; the emphasis has been on the effect of animal proteins on the kidney. Due to the high prevalence of renal disease in our society in recent times, and the fact that it is better to avoid having the disease, since treatment and management is very expensive, it is important to look at our lifestyle, especially our diets to ensure that what we consume is not a predisposing factor to kidney disease. It is therefore the aim of this study, to look at the effect of both plant (cowpeas) and animal (casein) proteins as high protein diets on renal morphology.

MATERIALS AND METHOD

Number of rats /grouping

One hundred and seventy wistar rats were used for this study; the rats were divided into three groups of A, B and C. Groups A and B had 80 rats in each group, which was further divided into subgroups of 20 per subgroup. A1 (25% cowpeas diet), A2 (30% cowpeas diet), A3 (35% cowpeas diet) and A4 (40% cowpeas diet) and group B were divided into subgroups of B1 (25% casein diet), B2 (30% casein diet), B3 (35% casein diet) and A4 (40% casein diet). Group C (10) was the control group; the rats were fed the normal rat chows (15% protein). The rats were allowed to acclimatize for two weeks before administration of experimental diets. Rats were housed in the Department of Pharmacology animal house in wooden cages according to their groups. They were allowed free access to food and water. Weights of the rats were taken at the beginning of the study; at 1 month; 3 month and 6 months prior to collection of samples.

Preparation of Diet

Cowpea: This was bought in our local market, cleaned and oven dried at 100^{o}Cfor 45 minutes to remove the anti nutritional factors [7]. The oven heated cowpeas was then blended and added to the other constituents of the diet. For the casein diet, casein powder was bought and used to constitute diet B. The proximate composition of the diet is as shown in table 1 and 2 below.

ANALYSIS

At the point of sample collection, 5 rats from each sub group (casein group, cowpeas group and control) were sacrificed and the right kidney removed for histological analysis. As soon as the rats were sacrificed, the kidneys were put in separate container containing 10% formal saline (sodium chloride, 40% formaldehyde and distilled water) for fixing the tissue. Dehydration, clearing, infiltration and embedding were done manually. The prepared sections were then stained with haematoxylyin and eosin staining technique[8]. Light microscope (x400) was used to view the prepared slides and then micrograph of the slides was taken.

RESULTS

Micrograph of the kidney stained with haematoxylin and eosin stains for control shows the glomeruli A, tubules B, interstitial space C as attached in plate 1. Rats fed 25%, 30 %, 35% and 40% casein diet at 3 and 6 months showed various morphological changes such as increased glomerular cell mass, focal tubular epithelial hyperplasia (plate 2a), oedematous tubular epithelium, hypertrophied arteriole (plate 3a), as well as the presence of mild chronic inflammatory cell infiltrates in rats fed 40% casein diet for 6 months (plates 4a and 5a). While rats fed cowpeas diets showed mild morphological changes such as oedematous tubular epithelium and hyperplastic tubular epithelium (plate 2b, 3b 4b and 5b).

Table 1: **PROXIMATE COMPOSITION OF EXPERIMENTAL DIET A (100%)**

Constituents	C (15%)	A1 (25%)	A2 (30%)	A3 (35%)	A4 (40%)
Protein (%)	**15**	**25**	**30**	**35**	**40**
Carbohydrate	47.19	39.95	32.26	26.45	20.55
Moisture	10.38	6.70	6.92	7.25	7.17
Fiber	16.35	15.35	15.32	15.25	15.38
Fat/oil	5.0	9.9	10	9.8	10.3
Ash	6.08	3.10	5.5	6.25	6.60

Table 2: **PROXIMATE COMPOSITION OF EXPERIMENTAL DIET (B)**

Constituents	C (15%)	B1 (25%)	B2 (30%)	B3 (35%)	B4 (40%)
Protein (%)	**15**	**25**	**30**	**35**	**40**
Carbohydrate	47.19	31.66	26.47	22.07	16.89
Moisture	10.38	9.54	9.56	9.55	9.63
Fat/oil	5.0	10.01	10	9.8	10.4
Fiber	16.35	17.0	17.22	17.23	17.25
Ash	6.08	6.79	6.75	6.35	5.83

MICROGRAH OF THE KIDNEY

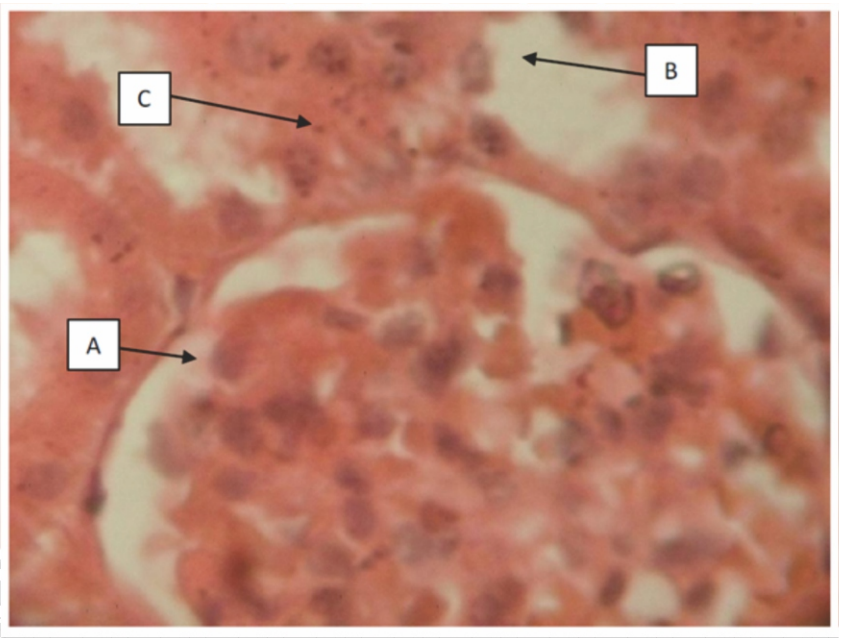


Plate1: Control: Rat kidney composed of glomeruli A, tubules B, interstitial space C (H&E x400)

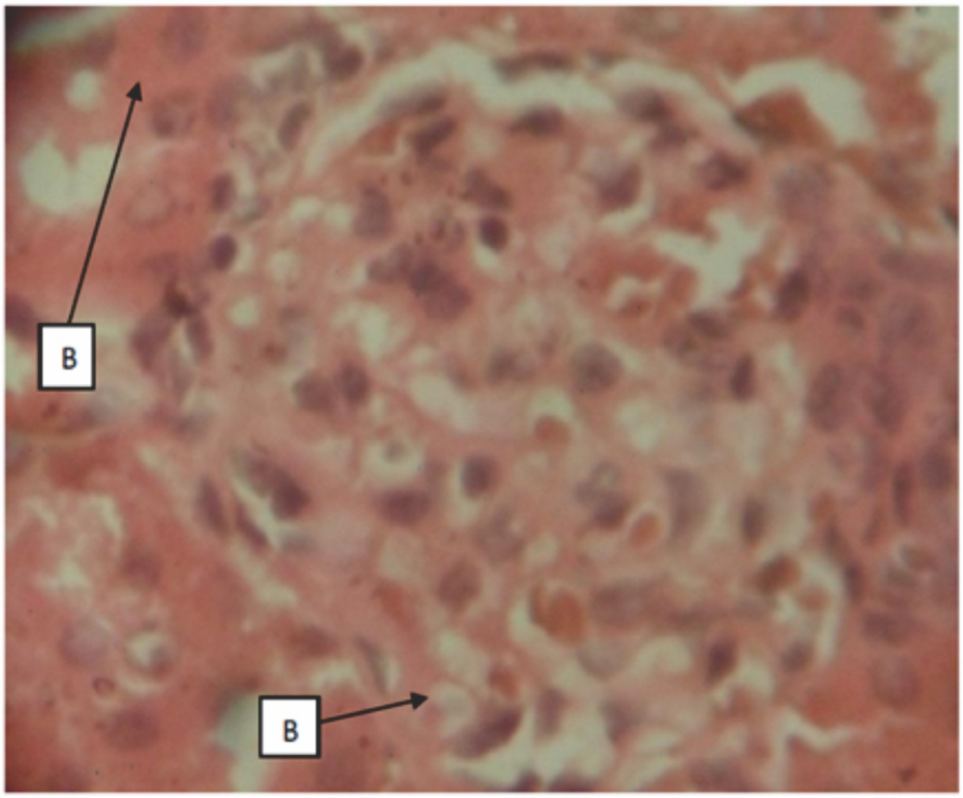


Plate: 2a Rat kidney given 25% casein for 3 months showing focal increased glomerular cell mass A and focal tubular epithelial

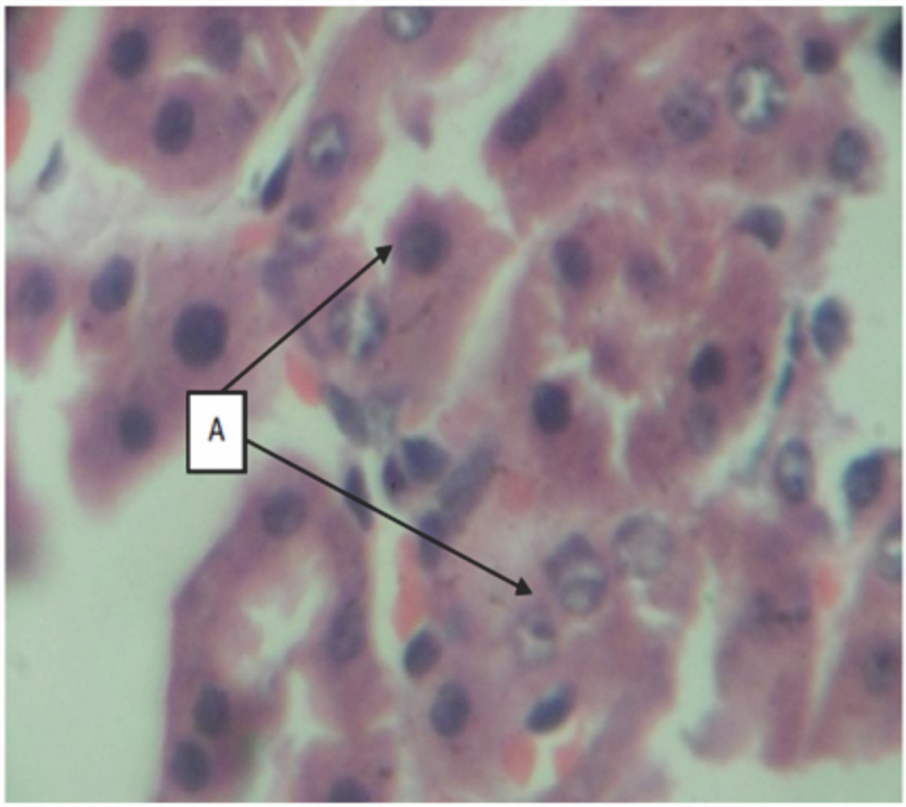


Plate 2b: Rat kidney given 25% cowpea for 3 months showing focal oedematous tubular epithelium A (H&E x 400)

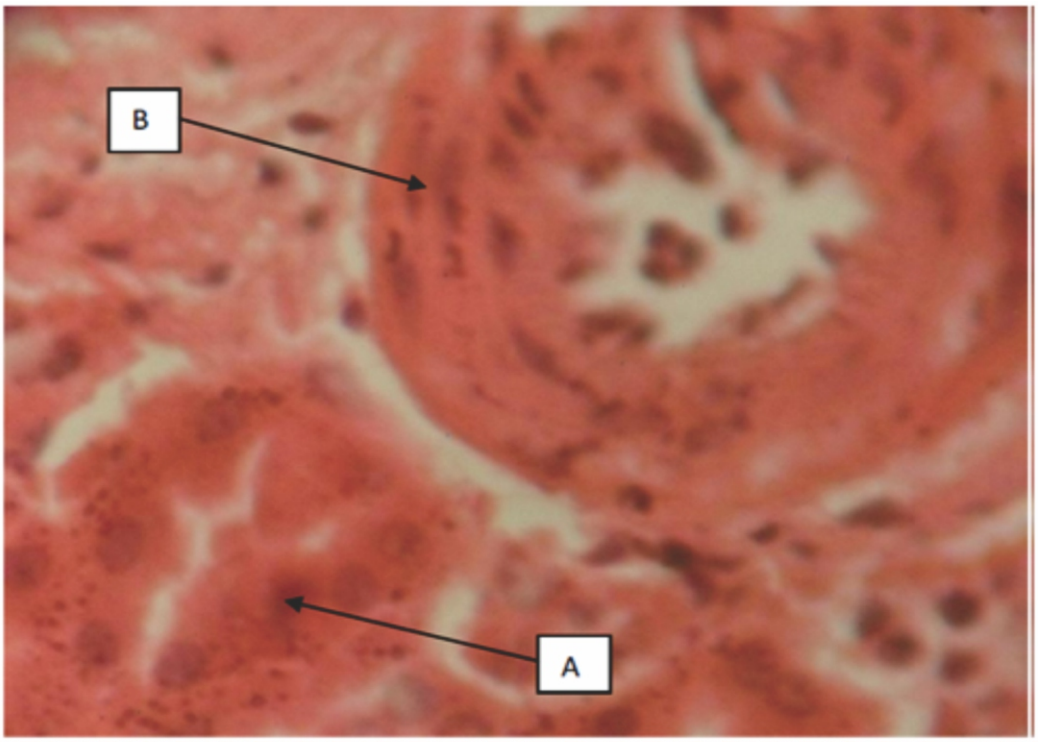
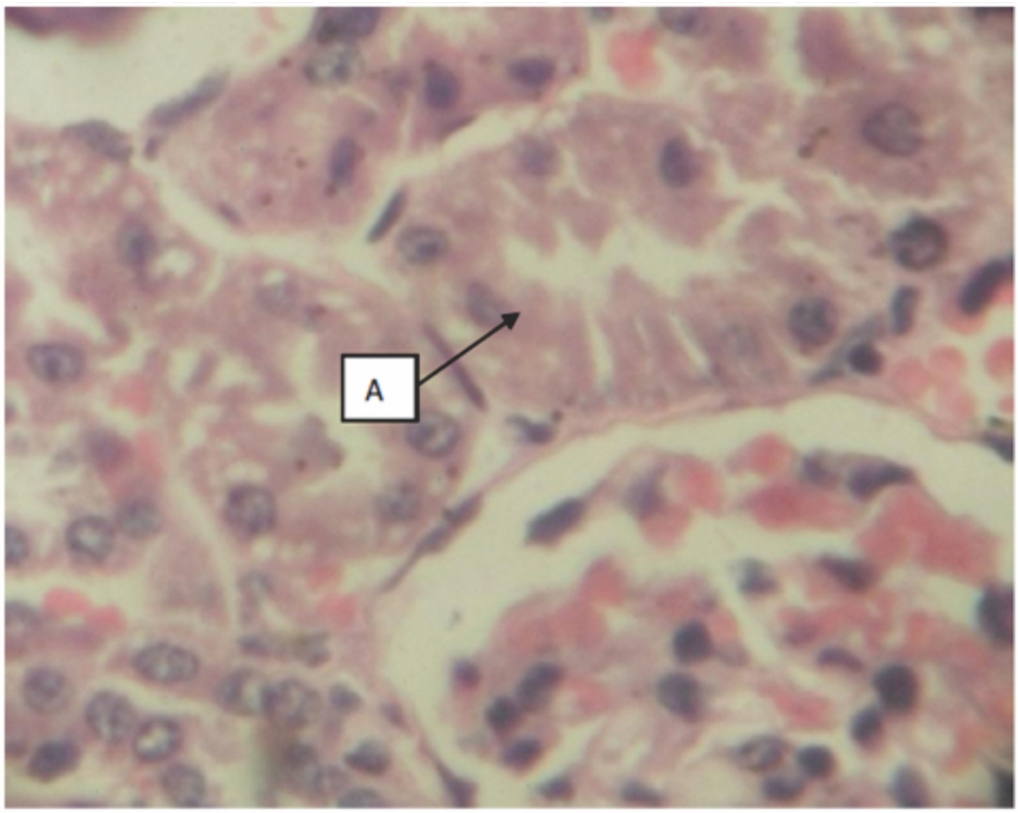


Plate 3a: Rat kidney given 30% casein for 3 months showing oedematous tubular epithelium (necrosis) A and hypertrophied arteriole B



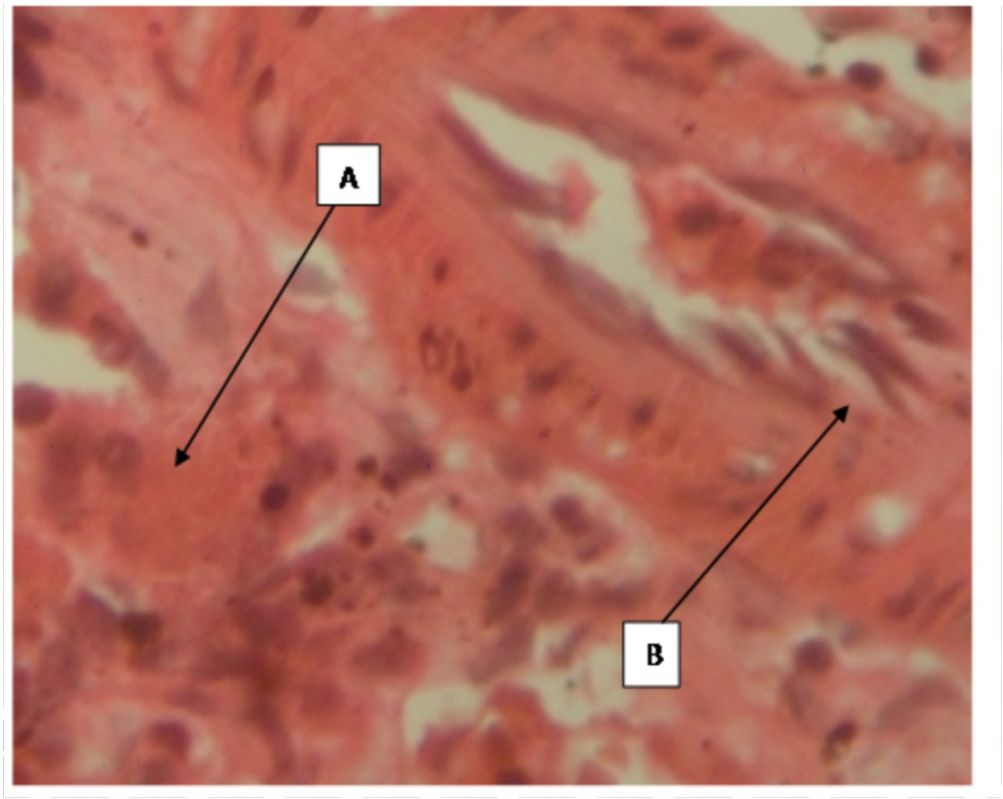


Plate 5a: Rat kidney given 40% casein for 3 months showing oedematous tubular epithelium (necrosis) A and hypertrophied

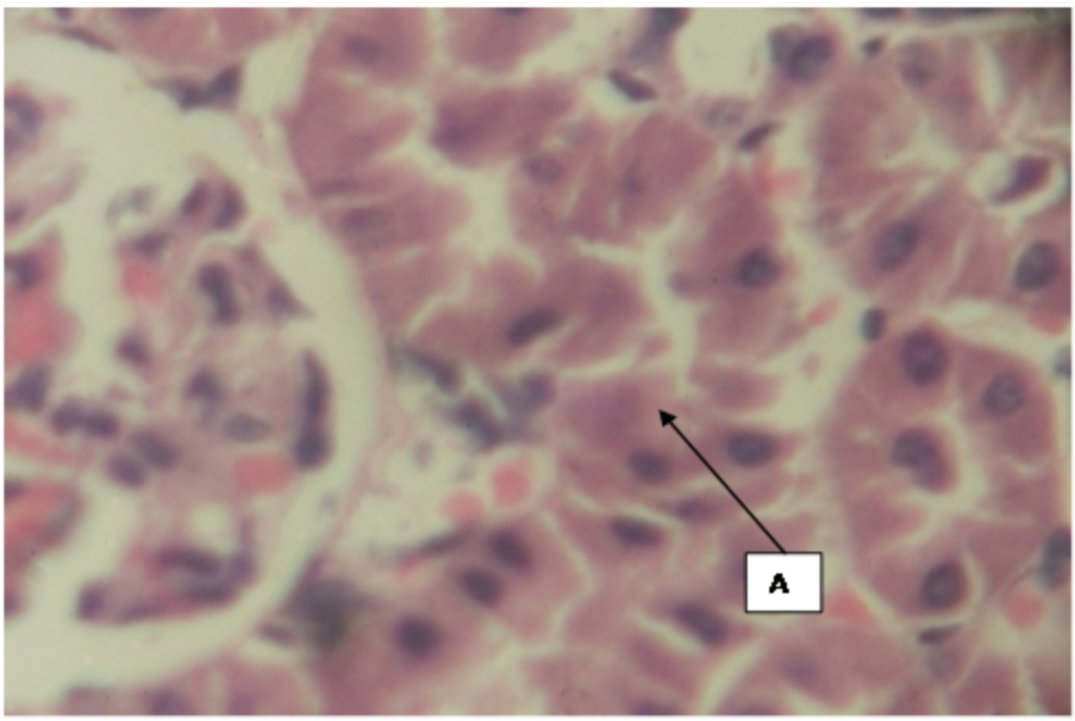


Plate 5b: Rat kidney given 40% cowpea for 3 months showing oedematous tubular epithelium A (H&E x 400)

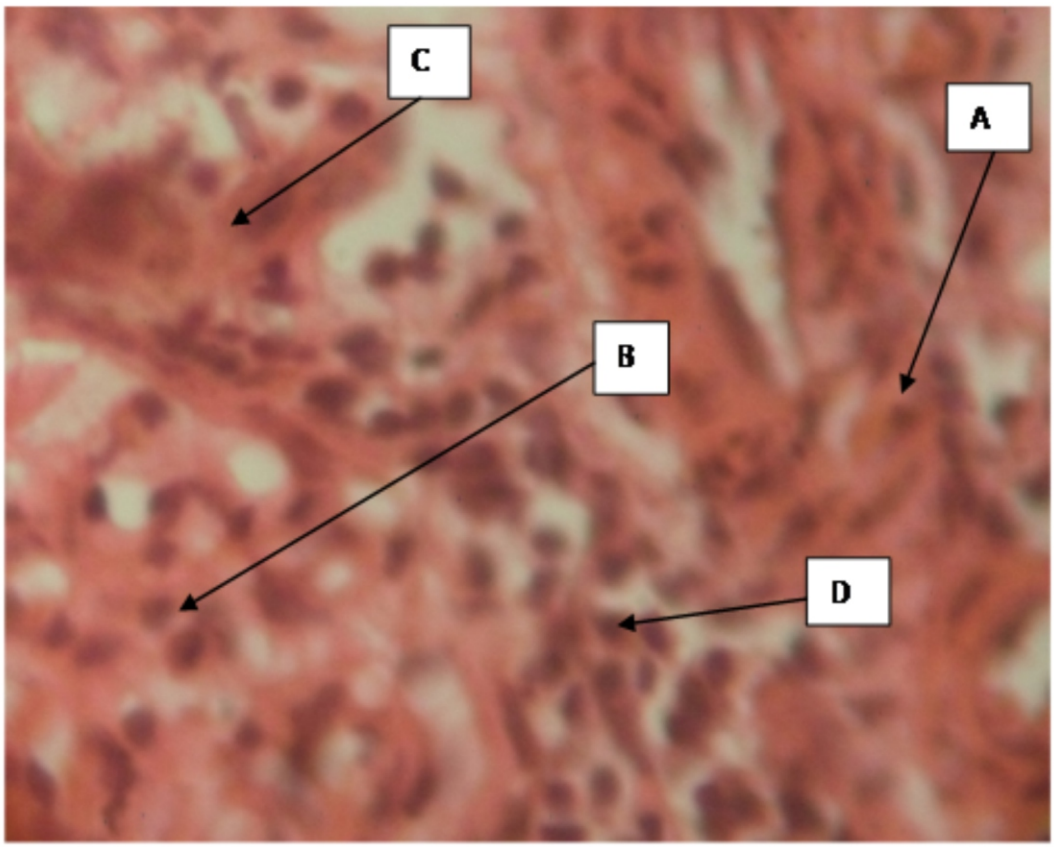


Plate 6a: Rat kidney treated with 40% casein for 6 months showing hypertrophied arteriole A, focally increased glomerular cell mass, B tubular cell hyperplasia C and mild chronic inflammatory cell infiltrates D (H&E x 400)

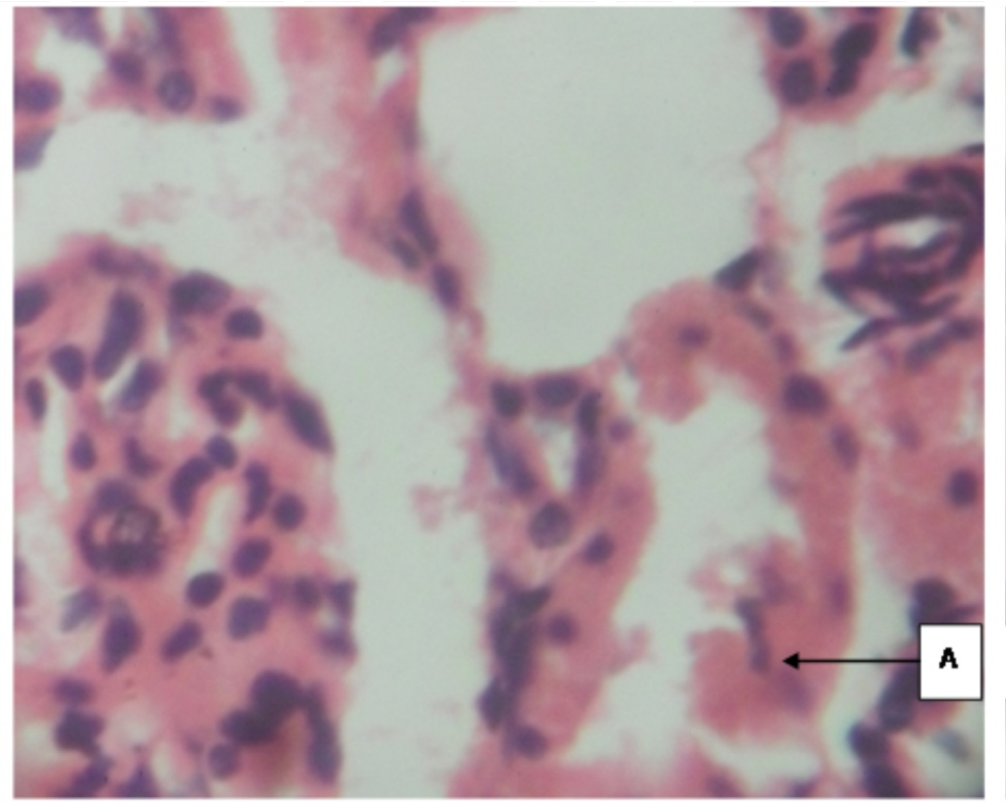


Plate 6b: Rat kidney given 40% cowpea for 6 months showing mild tubular oedema A (H&E x400).

DISCUSSIONS

Morphological changes were observed in rats fed graded levels (25%, 30%, 35% and 40%) of casein diet for 3 and 6 months respectively. Varied types of damages (increased glomerular cell mass, hyperplasia, necrosis and hypertrophied arteriole) in renal tubular epithelium, glomeruli and arteriole were observed. These changes were progressive in severity with increasing levels and duration. In the tubules, there were cloudy swellings which are evidence of tubular necrosis (plate 4a). There were also hypertrophied arteriolar walls and endothelia injury both of which resulted in narrowing and obstruction of the arterial lumen, which may have resulted in reduced blood flow (ischemia). This ischemia is the probable cause of tubular necrosis. In the glomerulus, the changes were mild, the most notable being the 40% casein for 6 months where there were infiltrates of lymphocytes and plasma cells and increase in glomerular cell mass (plate 5a). Lymphocytes and plasma cells are known to differentiate into antibodies and glomerular lesions and are known to be immune mediated, thus these cells of the immune system (lymphocytes and plasma cells) were probably activated by chronic administration of casein which may lead to the increase in glomerular cell mass (Mesangial proliferation). Studies have provided evidence for adverse effect of high protein diets on the normal kidney. Rats and mice exposed to high protein diets have a greater prevalence of developing nephropathy, including glomerular hypertrophy, glomerulosclerosis, tubulo-interstitial fibrosis, tubular regeneration and chronic inflammatory cell infiltration as observed in some earlier studies[9, 10, 11]. However, cowpeas fed rats showed minimal changes which is not significant. Our findings are in agreement with many other studies, dietary protein intake induced renal hypertrophy in dogs[12], while in rats; intake of high protein diet may lead to renal hypertrophy [13, 14, 15]. An independent effect of increased protein intake on renal hypertrophy in mice has also been demonstrated [16]. Recent studies have also shown some relationship between dietary protein intake and morphological changes in the kidney. Goldstein &Plaga[3]andBouby*et al*[4] reported histological damage with high protein diet in rodents. In another study, administration of whole plant and animal proteins to pigs in proportions that mimicked human diets resulted in enlarged kidneys at both 4 and 8 months in the high protein diet [6], renal glomerular volumes were 60 – 70% higher by the end of the study[6]. The enlarged kidneys had greater evidence of histological damage, with 55% more fibrosis and 30% more glomerulosclerosis, Some morphological impairment in the high protein diet fed group were also observed, with a 32% higher renal mesangium area, 30% higher floccules areas and 13% higher glomerular area but without significant more renal interstitial connective tissue. One of the first histological lesions observed is hyperplasia of the tubular epithelium which is presumed to be a mere compensatory phenomenon resulting from changes in the filtering ability of the glomerular turf[6].

Renal insufficiency, progressive proteinuria and renal structural lesions were observed in rats fed 20% casein diet for a period of 160 days while those fed on soy protein, had improved renal functions[17]. This is similar to findings in this present study as explained above. However, there are some studies which reported otherwise. No adverse effects of long term consumption of high protein diet on renal function was observed when two years of a diet containing 60% protein failed to evoke changes in the percentage of sclerotic glomeruli in rats [18]. In another study, there was no association between diet and structural changes in the kidney of dogs that were 75% nephrectomized, after four years of feeding diets that were either 56, 27 or 19% proteins [19].

CONCLUSION

The morphological changes observed in the kidney of rats in this study could be due to the effect of the diets on the kidney, considering the fact that in this study every other variable were same for both experimental groups and control except for the diet. We thus conclude that intake of high casein diet; an animal protein could be injurious to the kidney, however we recommend that more detailed studies should be done on the intake of high protein diets on the kidney using early markers along with morphological changes.

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