



## Carbendazim alters kidney morphology, kidney function tests, tissue markers of oxidative stress and serum micro-elements in rats fed protein-energy malnourished diet

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### ABSTRACT

The effects protein-energy malnourishment on the kidney morphology, kidney tissue markers of oxidative stress, tissue toxicity and plasma micro-elements in carbendazim-treated adult male Wistar rats were evaluated. Forty rats were divided into 4 groups fed low (4.11%) or normal (17.78%) protein-energy diets (LP and NP, respectively) and orally administered 200 mg carbendazim/kg body weight (LPC; NPC)/day for 14 days. Control groups received oil (LPO; NPO). Carbendazim reduced the relative body weight gains of the rats relative to the NPO, independent of their protein-energy diet status. Significant elevations ( $p < 0.05$ ) in the profiles of MDA, GSH, SOD, total bilirubin, conjugated bilirubin, urea, Ni, Se and lead were observed in the LPC group relative to the NPO group. The NPC group showed significantly decreased ( $p < 0.05$ ) levels of MDA and Zn relative to the NPO. However, the concentrations of CAT, GSH, total bilirubin, unconjugated bilirubin, Se and As were significantly elevated in the NPC relative to the NPO groups. Although histomorphological changes were observed in the kidney of the carbendazim-treated group on normal protein diet, protein-energy malnutrition did not exacerbate lesions which were contrary to tissue MDA which was elevated in LPC. The alterations in the profiles of tissue markers of oxidative stress, kidney function tests and some micro-nutrients observed in the kidney suggest that the toxic effects of carbendazim is more pronounced in the kidney of protein-energy malnourished rats.

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**Keywords:** Carbendazim, protein-energy malnourishment, kidney toxicity, micro-elements.

### INTRODUCTION

Carbendazim is a commonly used industrial fungicide with broad spectrum antifungal property and is used in the control of fungal pathogen in cereal and fruit crops such as cocoa (Mahob et al., 2014) Carbendazim and other benzimidazole

derivatives are used also as preservative in the paint, textile, papermaking - and leather industries (Selmanoglu et al., 2001). There are some evidence that the route of exposure of man and animals to carbendazim is via consumption of grains and fruits treated with the fungicide. Carbendazim is well absorbed

(80-85%) after oral exposure and it is subsequently metabolized into many compounds within the organism. Chronic intake and/or exposure to low doses of CBZ may exert some toxic effects. Carbendazim shows minimal toxicity with LD<sub>50</sub> > 15 g/kg (Shui-Yan et al., 2004), this low toxicity along with its antifungal effect has led to the suggestion that it might be useful in cancer chemotherapy (Gupta et al., 2004). The main metabolites are 5-hydroxy-2-benzimidazole carbamate and 5,6-HOBC-N-oxides (5,6-HOBC-N-oxides). Carbendazim and the metabolites, are poorly catabolized but are retained in tissues such as gonads, liver, adrenals, adipose, skin and other organs. The retained metabolites in tissues have been shown in laboratory animals to cause infertility and destruction of testicles *in vivo* (Lutz, 2012; Dikić et al., 2012). Carbendazim in pregnant rats has been shown to induce marked reproductive toxicity and developmental abnormality and has been found to disrupt microtubule formation by binding to tubulin (Lu et al., 2004). Also evidence abounds in experimental animals on the toxic effects of the fungicide as a hepatotoxicant (Barlas et al., 2002; Shui-Yan et al., 2004).

Malnutrition is a public health problem most common in developing countries. Protein-energy malnutrition is responsible for kwashiorkor, the most prevalent form of malnutrition especially in children. This is because protein foods are generally more expensive compared with other food nutrients. Low protein diet causes changes in metabolism in animals and this is proportional to the level of protein depletion and the length of time of malnutrition (Nwozo et al., 2016). The kidney is one of the most metabolically active organs in the body (Bimenya et al., 2008). Many biologically active molecules are either synthesized or degraded in the kidney. The molecules include amino acids, peptide hormones, other peptides, glucose and fatty acids. The impact of protein-energy

malnutrition on carbendazim-exposed rodents has not been documented; hence this study was designed to evaluate the impact of carbendazim on key indices of nephrotoxicity in rats fed either low protein or normal protein-energy diets.

## MATERIALS AND METHODS

### Chemicals

Carbendazim was obtained from Sigma-Aldrich Co. (St Louis, MO, USA). Bovine serum albumin (BSA) was from Sigma Chemical (St Louis, MO, USA). Diagnostic Kits for determination of serum  $\gamma$ GT, creatinine, albumin, bilirubin and urea were procured from Boehringer Mannheim Diagnostic (Mannheim, Germany). Other reagents used were of the purest quality commercially available.

### Feed formulation

The protein-energy diets used in this study were formulated as described by Akingbemi et al. (1994). Briefly, normal protein-energy diet (NP) was prepared using fish meal (90 g), whole maize flour (*Zea mays*) (420 g), maize starch (420 g), palm oil (60 g) and salt/vitamin mix (10 g). Same ingredients were used for low protein-energy diet (LP) except fish meal was 20 g and maize starch 490 per kg diet. The mineral mix, composed of Vitamin A 15,000,000 iu, vitamin D3 4,400,000 iu, vitamin E 1,350 iu, vitamin K 4,350 mg, vitamin B2 4,350 mg, vitamin B6 2,350 mg, vitamin B12 11,350 mg, vitamin C 1,000 mg, nicotinamide 16,700 mg, calcium pantothenate 5,350 mg, sodium sulphate 212,000 mg, KCl 87,000 mg, MgSO<sub>4</sub> 12,000 mg, ZnSO<sub>4</sub> 12,000 mg, MnSO<sub>4</sub> 12,000 mg, Lysine hydrochloride 15,000 mg, Methionine 10,000 mg, Excipients QS 1,000 mg, distributed in Nigeria by Polons Agro Investment Limited (www.anupco.com), and the different components of the diet were thoroughly mixed, made into pellets for ease of handling by animals, oven dried to prevent mold

growth and stored in air tight bags to prevent microbial contamination and auto-oxidation of the oil (Akingbemi et al., 1994).

#### Proximate analysis of the feeds

Feeds were analyzed for ash and mineral content using muffle furnace at 550 °C for 4 hrs. Moisture content was determined by drying in the oven at 100 °C until a constant weight was obtained (at least 24 hrs). Total dietary fiber was determined by an enzymatic gravimetric method, crude oil content was assayed by extraction with *n*-hexane in a Soxhlet extractor and nitrogen was determined by standard micro Kjeldahl method using a digestion apparatus (AOAC, 2005). The crude protein content was thereafter calculated by multiplying nitrogen content by a factor of 5.71, which takes into account the non-protein nitrogen. Moisture content was determined by drying to a constant weight at 100 °C for 24 hrs.

#### Experimental animals

Forty adult male albino rats of Wistar strain, weighing between 150 - 185 g, were obtained from Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, and were used in this study. The rats were acclimatized for two weeks in the Biochemistry Department Animal House in well ventilated plastic cages under normal room temperature with a 12-hour light and dark cycle before the commencement of the experiment and were allowed access to drinking water and normal rat chow (Ladokun Feed) *ad libitum*.

#### Experimental design

After the two weeks acclimatization period, 40 rats were distributed randomly into four groups of ten animals each. Corn oil was the vehicle used to administer carbendazim at dose of 200 mg/kg body weight of rat (Balkan and Altac, 2005). Group 1 (LPO) received 0.38 mL of corn oil (O) and low protein diet (LP). Group 2 (LPC) received carbendazim (C) and

LP; Group 3 (NPO) received 0.38 mL of corn oil (O) and normal protein (NP) diet. Group 4 (NPC) rats were treated with carbendazim and NP diet. Treatment duration was for 14 days, after which the animals were anaesthetized, bled and sacrificed.

#### Sample collection

After the day 14, all the animals were weighed, anaesthetized with light diethyl ether and blood samples were collected by venipuncture into both heparinized sample bottles and anticoagulant-free sample bottles. Blood samples were centrifuged at 3000 g for 10 mins in a MSC bench centrifuge (Beckman and Hirsch, Burlington, IO, USA) and both the plasma and the serum samples were collected. Plasma was used for metal analysis and the serum was used for markers of tissue toxicity. Kidneys were excised from each animal and weighed. One of the kidneys from each animal was rinsed in ice-cold 1.15% KCl and stored until required for further tissue antioxidant assays. The remaining kidney was fixed in 10% phosphate-buffered neutral formalin, processed routinely through graded ethanol and then paraffin wax. Sections, 5-8 µ thick, were cut and stained with haematoxylin and eosin for histopathological examination and photomicroscopy. Plasma micro-elements were analyzed at Central Laboratory, University of Ibadan using atomic absorption spectrophotometry while kidney function tests were performed on the serum samples.

#### Biochemical assays

Tissue lipid peroxidation was assayed by measuring thiobarbituric acid reactive substances (TBARS), by colorimetric reaction of the lipid peroxidation product malondialdehyde (MDA) with thiobarbituric acid to form a pink precipitate, which was read at 532 nm by spectrophotometry. Catalase (CAT) activity was done by measuring the rate of decomposition of hydrogen peroxide at 570 nm as described by

Sinha (1971). Reduced glutathione (GSH) level was determined by measuring the rate of formation of chromophoric product in a reaction between DTNB (5,5'-dithiobis-(2-nitrobenzoic acid) and free sulphhydryl groups at 412 nm. Superoxide dismutase (SOD) activity was assayed using the method of Misra and Fridovich (1972), Gamma glutamyltransferase activity was measured based on the transfer of glutamyl peptide to an amino acid of another peptide, glycylglycine to yield cleavage product p-nitroanilide which is measured at 405 nm. Total protein was determined using method of Gornal et al. (1949) and Bovine serum albumin as standard. Serum albumin level was determined using bromocresol green (Doumas et al., 1997). Bilirubin was determined using assay kit by diazotizing plasma bilirubin to form purple azobilirubin using sulphanilic acid (Ehrlich's reagent). Total bilirubin was determined in the presence of caffeine which releases albumin bound to bilirubin by the reaction of diazotized sulphanilic acid and the absorbance measurement was taken at 578 nm. Creatinine concentration was determined using commercially available Randox kit based on the reaction of creatinine in alkaline solution with picric acid to form colored complex which can be measured at 490-510 nm. Serum urea determination was done spectrophotometrically by diacetylmonoxime using thiosemicarbazide at 546 nm.

#### Statistical analysis

All values were expressed as the mean  $\pm$  SEM. Data were analyzed using one-way analysis of variance (ANOVA) followed by the post-hoc Duncan multiple range test for analysis of biochemical data using SPSS (10.0) statistical software. P values < 0.05 were considered statistically significant.

#### RESULTS

Data obtained for body weight of experimental animals are shown on Table 1. Generally, NP increased relative weight gain

while LP decreased the weight gain. Carbendazim treatment independently decreased the weight gain. There was a significant decrease in body weight gain in LPC compared to NPC and the LPO versus the NPO group of rats. In all the treatment groups, there was observed decrease in body weight during the duration of this study and only in NPO was the decrease mild. There was a significant increase ( $p < 0.05$ ) in the weight of kidneys in the LPC and NPC compared to the LPO and NPO (Table 1). Feed was significantly higher in NPO relative to LPO and NPC and also in NPC compared with LPC and NPO.

The concentrations of CAT and MDA were influenced by dietary status of the rats, been decreased by NP and increased by the LP (Table 1) while reversal of the effects of diets were observed in the concentrations of GSH and SOD. Carbendazim treatment alone increased the levels of GSH and SOD (Table 1). Rats that received LP and Carbendazim concurrently showed increased level of MDA (decreased in the presence of NP) and decreased level of CAT (increased in the presence of NP). The activities of MDA, SOD and CAT were significantly increased ( $p < 0.05$ ) while that of GSH significantly decreased ( $p < 0.05$ ) in the LPC relative to the NPO (Table 1). However, the activities of CAT and GSH were significantly increased, MDA being significantly decreased ( $p < 0.05$ ), in the NPC when compared with the NPO.

The effects of carbendazim and protein-energy diets on the serum gamma-glutamyltransferase ( $\gamma$ GT), creatinine, urea, total bilirubin, conjugated and unconjugated bilirubin are as shown on Table 2. There were slight elevations in the serum  $\gamma$ GT levels of rats exposed to carbendazim, irrespective of the dietary protein-energy status of such rats. Also carbendazim administration caused significant increases in the serum urea and creatinine levels. There were also significant increases in the total, conjugated and

unconjugated bilirubin in the LPC and NPC groups.

Results for essential trace element and some toxic heavy metals in the plasma are as shown on Table 3. The toxic metals were generally higher in the carbendazim-treated rats which consumed either LP or NP diets. Specifically, Arsenic level was significantly increased in NPC compared with LPC and NPO, while Pb and Cd levels were highest in the LPC compared with the other treatment

groups. There was a significant decrease in Se level and significant increase in Pb, Cd, Zn, Ni and As levels in the LPC and NPC groups.

Figures 1(a-d) show photomicrographs of kidney of rats in this study. There were no observable lesions in the kidneys of LPO, LPC and NPO groups. However, there was mild necrosis with presence of hyaline cast in the lumen of the kidney of animals on NPC when compared to NPO with normal kidney tissue morphology.

**Table 1:** Effect of carbendazim and varying protein-energy diets on body weight, kidney weight, protein concentration, lipid peroxidation, reduced glutathione, superoxide dismutase and catalase activities in the kidney of male rats.

Parameters	LPO	LPC	NPO	NPC
Body weight increase (%)	-10.14±1.1	-14.25±1.3 <sup>a</sup>	-0.98± 0.009 <sup>bc</sup>	-6.73±0.07 <sup>def</sup>
Kidney weight (g)*	0.895±0.07	1.05±0.071 <sup>a</sup>	0.715±0.07 <sup>bc</sup>	1.15±0.071 <sup>def</sup>
Feed intake	152.0±9.092	139.5±15.416	192.5±6.455 <sup>bd</sup>	184.750±.85 <sup>cc</sup>
KP concentration (mg/ml) *	1.403±0.006	1.233±0.072	3.827±0.075 <sup>b</sup>	1.457±0.6
MDA (Units/mg protein) *	1.203±0.14	1.301±0.215	0.444±0.094 <sup>bc</sup>	0.132±0.013 <sup>def</sup>
SOD (unit/mg protein) *	1.867±0.231	3.6±1.217 <sup>a</sup>	2.6±0.721 <sup>c</sup>	4±0.0 <sup>df</sup>
CAT (unit/mg protein) *	524.5±51.8	469.4±21.7	177.1±5.9 <sup>bc</sup>	476.2±33.3 <sup>df</sup>
GSH (µg/ml) *	0.004±0.002	0.006±0.002 <sup>a</sup>	0.008±0.001 <sup>bc</sup>	0.013±0.003 <sup>def</sup>

Results are presented as mean ± SEM (n=10); \*p<0.05. KP (Kidney protein).

LP (Low protein-energy diet); NP (Normal protein-energy diet); C (Carbendazim/Cbz.); O (Oil).

<sup>a</sup>LPO versus LPC (Cbz. effect); <sup>b</sup>LPO versus NPO (Diet effect); <sup>c</sup>LPC versus NPO;

<sup>d</sup>LPO versus NPC; <sup>e</sup>LPC versus NPC (Cbz.-diet interaction); <sup>f</sup>NPO versus NPC (Cbz. effect).

**Table 2:** Effect of protein-energy malnutrition and carbendazim on serum γ-GT, urea, creatinine and bilirubin levels in the adult male Wistar rat.

Parameters	LPO	LPC	NPO	NPC
Total bilirubin (nm/min)*	4.28±0.66	8.78±0.86 <sup>a</sup>	3.79±0.5 <sup>c</sup>	7.79±1.11 <sup>df</sup>
Cg. bilirubin (nm/min)*	3.36±0.25	7.7±0.52 <sup>a</sup>	3.03±0.34 <sup>c</sup>	3.09±1.65 <sup>c</sup>
U. bilirubin (nm/min)*	0.543±0.066	0.76±0.249	0.467±0.04	0.833±0.09 <sup>df</sup>
γ-GT (U/L)	5.02±0.67	5.82±0.67	5.01±0.67	5.79±1.17
Urea (mg/dl)*	14.98±1.34	33.01±4.12 <sup>a</sup>	13.25±5.45 <sup>c</sup>	26.02±4.11 <sup>df</sup>
Creatinine (mg/dl)	0.163±0.054	0.185±0.023	0.161±0.024	0.186±0.042

Results are mean ± SEM (n=10); \*p<0.05. Cg. (Conjugated); U (Unconjugated).

LP (Low protein-energy diet); NP (Normal protein-energy diet); C (Carbendazim/Cbz.); O (Oil).

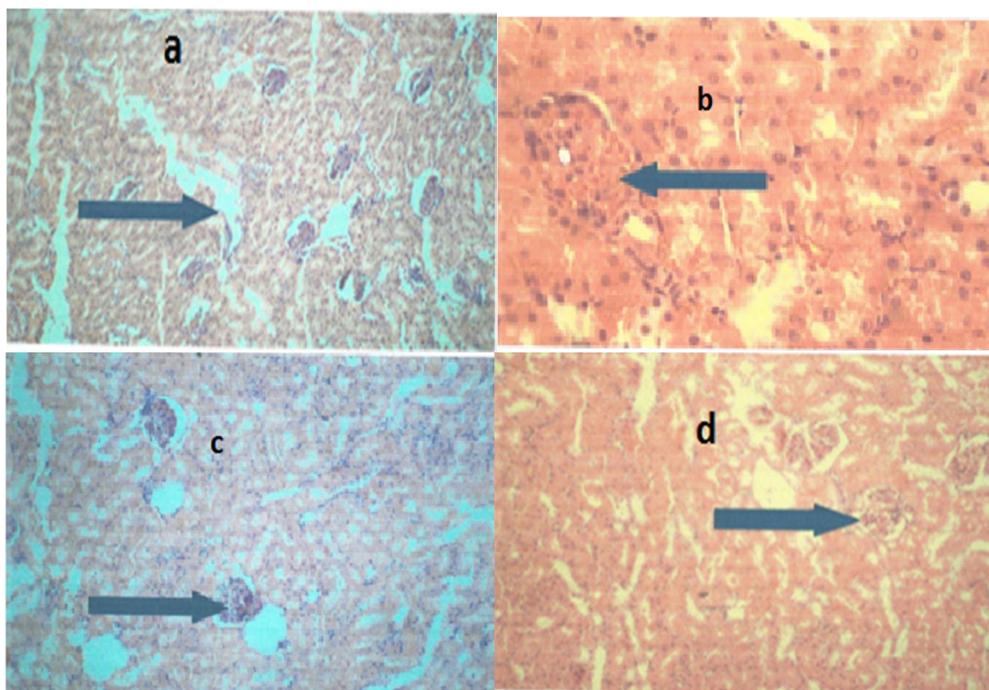
<sup>a</sup>LPO versus LPC (Cbz. effect); <sup>b</sup>LPO versus NPO (Diet effect); <sup>c</sup>LPC versus NPO;

<sup>d</sup>LPO versus NPC; <sup>e</sup>LPC versus NPC (Cbz.-diet interaction); <sup>f</sup>NPO versus NPC (Cbz. effect).

**Table 3:** Effects of protein-energy diets and/or carbendazim administration on the plasma levels of micro-elements ( $\mu\text{g}/\text{dl}$ ) in the adult male rat.

Micro-element	LPO	LPC	NPO	NPC
Ni***	2.298 $\pm$ 0.286	6.244 $\pm$ 0.096 <sup>a</sup>	2.847 $\pm$ 0.147 <sup>bc</sup>	3.074 $\pm$ 0.054 <sup>dc</sup>
Zn*	16.3 $\pm$ 1.4	20 $\pm$ 0.8 <sup>a</sup>	17.7 $\pm$ 2.6	10.1 $\pm$ 0.4 <sup>d<sup>ef</sup></sup>
Se*	0.007 $\pm$ 0.002	0.010 $\pm$ 0.001 <sup>a</sup>	0.001 $\pm$ 0.003 <sup>bc</sup>	0.008 $\pm$ 0.001 <sup>ef</sup>
Pb***	5.096 $\pm$ 0.031	13.893 $\pm$ 0.369 <sup>a</sup>	7.257 $\pm$ 0.245 <sup>bc</sup>	6.046 $\pm$ 1.673 <sup>c</sup>
Cd	0.132 $\pm$ 0.062	0.194 $\pm$ 0.007	0.153 $\pm$ 0.043	0.160 $\pm$ 0.005
As**	0.018 $\pm$ 0.002	0.021 $\pm$ 0.001	0.014 $\pm$ 0.002 <sup>bc</sup>	0.03 $\pm$ 0.002 <sup>d<sup>ef</sup></sup>

Results are presented as mean  $\pm$  SEM (n=10); \* p<0.05; \*\* p<0.01; \*\*\*p<0.001.  
 LP (Low protein-energy diet); NP (Normal protein-energy diet); C (Carbendazim/Cbz.); O (Oil).  
<sup>a</sup>LPO versus LPC (Cbz. effect); <sup>b</sup>LPO versus NPO (Diet effect); <sup>c</sup>LPC versus NPO;  
<sup>d</sup>LPO versus NPC; <sup>e</sup>LPC versus NPC (Cbz.-diet interaction); <sup>f</sup>NPO versus NPC (Cbz. effect).



**Figure 1:** (a-d) Show photomicrographs of kidney of rats in this study. a: LPO rat kidney showing no visible lesion; b: LPC rat kidney showing no visible lesion; c: NPO rat kidney showing no visible lesion; d: NPC rat kidney showing very mild tubular necrosis (with presence of hyaline casts in tubular lumen).

## DISCUSSION

Administration of carbendazim to rats on low and normal protein-energy diets elicited changes in biochemical parameters and the kidney histo-architecture in rats used in this study. There was also significant decrease in body weight of LPC animals compared with NPO. The decrease in body weight was significant in LPO compared with NPO and LPC compared with NPC. This observation was not surprising as there was also a significant decrease in feed intake in LPO and LPC compared to NPO and NPC. Carbendazim administration affected the absolute weights of kidney tissue (Table 1). Absolute weight of the kidney is a relatively sensitive predictor of nephrotoxicity (Seely, 2017). Enlarged kidney, from diseased state, could cause changes in metabolic and hormonal activity which could further cause reduced appetite and diminished nutrient intake (Kopple, 1999; Jean et al., 2001). The high depletion in protein concentration and decrease in relative body weight and reduction in feed intake in LPO and LPC could be due to both LP diet not being acceptable to rats compared with normal protein diet and carbendazim toxicity at the dose used in this study.

Malonyldialdehyde (MDA) is an index of tissue membrane degeneration via free radical production (Veena et al., 2007). MDA levels were significantly higher in carbendazim-treated rats fed different percentages of protein-energy diets for 14 days. The LPO group also had higher MDA than the NPO group suggesting that both carbendazim and LP diet induced the increased lipid peroxidation. It could, however, imply that NP diet had higher percentage of constituents with antioxidant properties than the LP diet. Increased MDA could also suggest membrane instability and might indicate oxidative stress. The antioxidant system plays a critical role in protecting tissues (Oschendorf, 1999).

The reductions in CAT activities in the kidney of carbendazim-treated groups could indicate elimination of hydrogen peroxide by

reactive oxygen, an observation similar to report on sulfasalazine-induced oxidative stress (Alonso et al., 2009). However there was increase in CAT in the same organ of rats fed NP. It has been observed that increased SOD is beneficial in event of increased free radical production (Pham-Huy et al., 2008). SOD was significantly increased in LPC compared with LPO in the kidney but decreased in NP. CAT increased in LPO compared with LPC but NPO had significantly decreases values compared with both LPC and LPO. GSH was found to be elevated in animals treated with CBZ in our study on both low and normal protein diets relative to LPO and NPO. These observations in SOD, CAT, GSH in the kidney indicate an imbalance in the antioxidant system in the animals, thus leading to increased peroxidation of membrane in the organs.

Elevated value of  $\gamma$ GT implies an increase in transpeptidases and this could have deleterious effects on tissue morphology as the enzyme is localized in the mesangial, podocyte and tubular epithelia cells of the kidney and is only released into cellular flow on tissue damage (Muthuviveganandave et al., 2011). Urea and creatinine are renal function markers and the increases observed in the urea and creatinine levels may be attributed to the synergistic effects of LP diet and carbendazim toxicity (Abolaji et al., 2016; Sarama and Padjama, 2013; Chu et al., 2008), thus decreasing the renal elimination of these metabolic waste.

The most prevalent form of protein-energy malnutrition leads to kwashiorkor, resulting from metabolic disorder, mal-absorption of nutrients, and loss of protein and could promote complications such as ascites, encephalopathy, infections and hepatorenal syndrome (Krawinkel, 2003). There was a significant increase in total, conjugated and unconjugated bilirubin in the LPC and the NPC groups while the NPO group had lowest values of all the three parameters. Elevated bilirubin could be a protective mechanism against organ damage from haemoglobin degradation. Bilirubin is metabolized by

bilirubin-UDP-glucuronyltransferase to produce more water soluble bilirubin diglucuronide derivative, thus facilitating biliary excretion. Thus, total bilirubin in this study could be not just the effective metabolism but also the excretion from the body. Oxidative stress occurred in both the LPC and the NPC groups, but it seems as if bilirubin produced has been channeled to combat free radical generation. Plasma bilirubin has been linked to increase in antioxidant capacity and reduced oxidative stress (Daniet al., 2003).

The levels of Zn and Ni, essential trace elements, were highest in LPC and in a similar manner Se was significantly increased in the carbendazim-treated rats. Trace metals are known to play important roles in the catalytic activities of major antioxidant enzymes. Zinc is found at the catalytic site of carbonic anhydrase and is an integral part of Cu-Zn SOD. Se is a co-factor in the active site of glutathione peroxidase (Se-GPX) which is a major scavenger of H<sub>2</sub>O<sub>2</sub>. We observed a significant decrease in Se and significant increase in Pb, Zn, Ni and As in the LPC and NPC groups. The decrease in Se could have resulted from the metal playing a role in combating oxidative stress and possibly diminished quantity in low protein feed could have contributory effect. Support for this could come from the observation that plasma Ni and Zn increased in LPC more than LPO and was higher in NPO than LPO. Earlier, it has been observed that deficiency of Se, Zn and Cu decreased activities of superoxide dismutase and glutathione peroxidase (Arinola et al., 2008) and this might be due to carbendazim which has been known to induce oxidative stress in animals. The presence of toxic heavy metals (Pb, Cd and As) was higher in animal exposed to carbendazim and this could have led to elevated levels in the plasma.

We have looked at the effect of varying percentage of protein diet on carbendazim-treated rats and in both NPC and LPC there was decrease in bodyweight, enlargement of kidney and elevation in renal markers such as

Gamma GT, creatinine, urea and bilirubin. Similarly toxic heavy metals were higher in carbendazim-exposed rats in both categories and had diminished values of antioxidative metals. Furthermore, kidney histopathological examination showed normal tissue morphology in groups on LPO, NPO and LPC at the dosage and duration of administration used in this study but there was very mild tubular necrosis in NPC-treated rats which incidentally had lower concentration of essential trace elements. This might imply that serum marker results alone should not be used as conclusive evidence of tissue damage as observed in our study.

#### COMPETING INTERESTS

The authors declared that there is no competing interest.

#### AUTHORS' CONTRIBUTIONS

SON and PCO designed the project, SON wrote up the paper, PCO read through the manuscript and OO did the laboratory work.

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