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Toxicological Evaluation of *Melocia corchorifolia* Leaves (L.) Fed to Albino Rat

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ABSTRACT

Wild leafy vegetables are widely eaten in developing countries and serves as nutrient supplements. The present study examined the effect of feeding albino rats with 75% *Melocia corchorifolia* leaves with respect to their body weight, liver and kidney biochemical, haematological and histological response. Results showed that the rats fed with *M. corchorifolia* leaves experience decrease in body weight compared to the control group. The Packed Cell Volume (PCV), haemoglobin concentration (Hb) and Red Blood Cells (RBC), White Blood Cells (WBC), platelets, Mean Carpuscular Volume (MCV), Mean Carpuseular Haemoglobin (MCH), Mean Carpuscular Haemoglobin Concentration (MCHC) and leukocyte (lymphocyte, neutophils, monocytes, eosinophils and basophils) differential counts were not significantly ($p>0.05$) different between control and treatment. Similarly, serum total protein, globulin and bilirubin were not significantly different, but that of albumin was significantly lower ($p<0.05$) in the treatment than control group. The serum enzyme activities, i.e., aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) were significantly ($p<0.05$) elevated in sample treatment than the control; which is an indication of organ toxicity by cellular destruction induced by the phyto-toxin present in the fed. Renal function indices-serum creatinine, urea, uric acid and electrolytes were not significantly different ($p>0.05$) between control and treatment. The results of this study showed that *Melocia corchorifolia* leaves have a relatively low or no toxicity profile.

Key words: Albino rat, toxicity, haematology, serum biochemistry, histology, *Melocia corchorifolia*, vegetables

INTRODUCTION

Wild leafy vegetables are widely eaten in developing countries not only during the period of food scarcity but also during period of abundance; perhaps due to cultural acceptance. Available literatures have shown that some wild leafy vegetables are rich in both macro-and micro-nutrients. For example, a study carried out in Brazil shows that *Pereskia aculeata* Miller leaves had 28.4% DW protein (Takeiti *et al*., 2009). Leaves of *Lycianthes synanthera*, *Spinacia oleracea*, *Lepidium sativum* and *Ipomea batatas* were reported to contain 36.0, 34.4, 24.5 and 29.5% of protein, respectively (Salazar *et al*., 2006; Ishida *et al*., 2000). Research conducted in Hausa land of Southern Niger and Northern Nigeria by Humphry *et al*. (1993) reported that wild plants from

the regions had considerable amount of protein. The report further indicated that leaves of *Sclerocarya birrea*, *Commiphora africana*, *Hibiscus canabinus*, *Cassia occidentalis*, *Ficus dekdekenna*, *Corchorus tridens* had protein content within the range of 11.00-30% on dry weight. *Adansonia digitata* leaf was also indicated to have protein content within the range of 10.1-15% (Glew *et al.*, 1997; Chadare *et al.*, 2009). *Moringa oleifera* leaf was also reported by Lockett *et al.* (2000) to have protein content of 20.72%. Cook *et al.* (1998) also reported high protein content (39.4%) in *Maerua crassifolia* leaf. From the data presented, it can be judged that wild plants can serve as food supplement to fight protein malnutrition. This can further be justified on study carried out in Senegal using *Moringa oliefera* leaves in the treatment of malnourish children. The result was impressive as within ten days positive result was seen compared to the combined use of whole milk powder, sugar, vegetable oils and peanut butter (Sreenivasan, 2000).

The carbohydrate content in wild leafy vegetables varies depending on the specie. Lockett *et al.* (2000) reported the carbohydrate content of some plants leaves of North eastern Nigeria to be within the range of 27-40% on DW. Humphry *et al.* (1993) reported the carbohydrate content of leafy vegetables found in Hausa land of Northern Nigeria to be up to 35-83%. Nordeide *et al.* (1996) reported the carbohydrate content of some leafy vegetables of Koutiala district of Mali as 39-73% DW. Leaves of *Tribulus terrestis*, *Gynandropsis gynandra* and *Momordica balsamina* were also reported to contain carbohydrates of 56% (Hassan *et al.*, 2005a), 43-59% (Hassan *et al.*, 2005b, 2007) and 39% (Hassan and Umar, 2008), respectively. Based on this, it can be concluded that leafy vegetables could be an important source of dietary energy.

The fibre content has been reported to have beneficial effects on blood cholesterol and aids in the prevention of large bowel diseases and improved glucose tolerance in, diabetic patients (Hart *et al.*, 2005). Wild leafy vegetables are also good sources of dietary fibre (Kala and Prakash, 2004, 2006; Gupta *et al.*, 2005).

Wild leafy vegetables are also rich sources of minerals and vitamins justify by numerous reported work (Yazzie *et al.*, 1994; Glew *et al.*, 1997; Nordeide *et al.*, 1996; Cook *et al.*, 1998, 2000; Udosen *et al.*, 1999; Hassan *et al.*, 2005a, b, c; Hassan and Umar, 2006, 2008).

Even though wild leafy vegetables are important sources of the above mention nutrients, less attention was given to the study of their toxic effects. Injection of some phytocompounds may lead to hepatic or/and tubular necrosis. This study aimed at investigating the toxic effect of *Melocia corchorifolia* leaves using experimental animals.

MATERIALS AND METHODS

Samples collection and transportation: Tender leaves of *Melocia corchorifolia* were randomly sampled from different locations along River Zamfara at Jega, Kebbi State (Fig. 1). Prior to analyses, the samples were identified and authenticated at the Herbarium of the Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria. The leaves were separated from the stalk, washed with distilled water, put in separate large study envelopes and oven dried at 60°C to constant weight (Fasakin, 2004). The dried leaves were pulverised in a porcelain mortar, sieved through 20-mesh sieve and stored in plastic containers. The powdered samples were used for the analyses.

Toxicity study

Experimental animals and feed preparation: Ten female albino rats weighing between 140-145 g used in the study were obtained from Faculty of Pharmaceutical Sciences, Ahmadu Bello

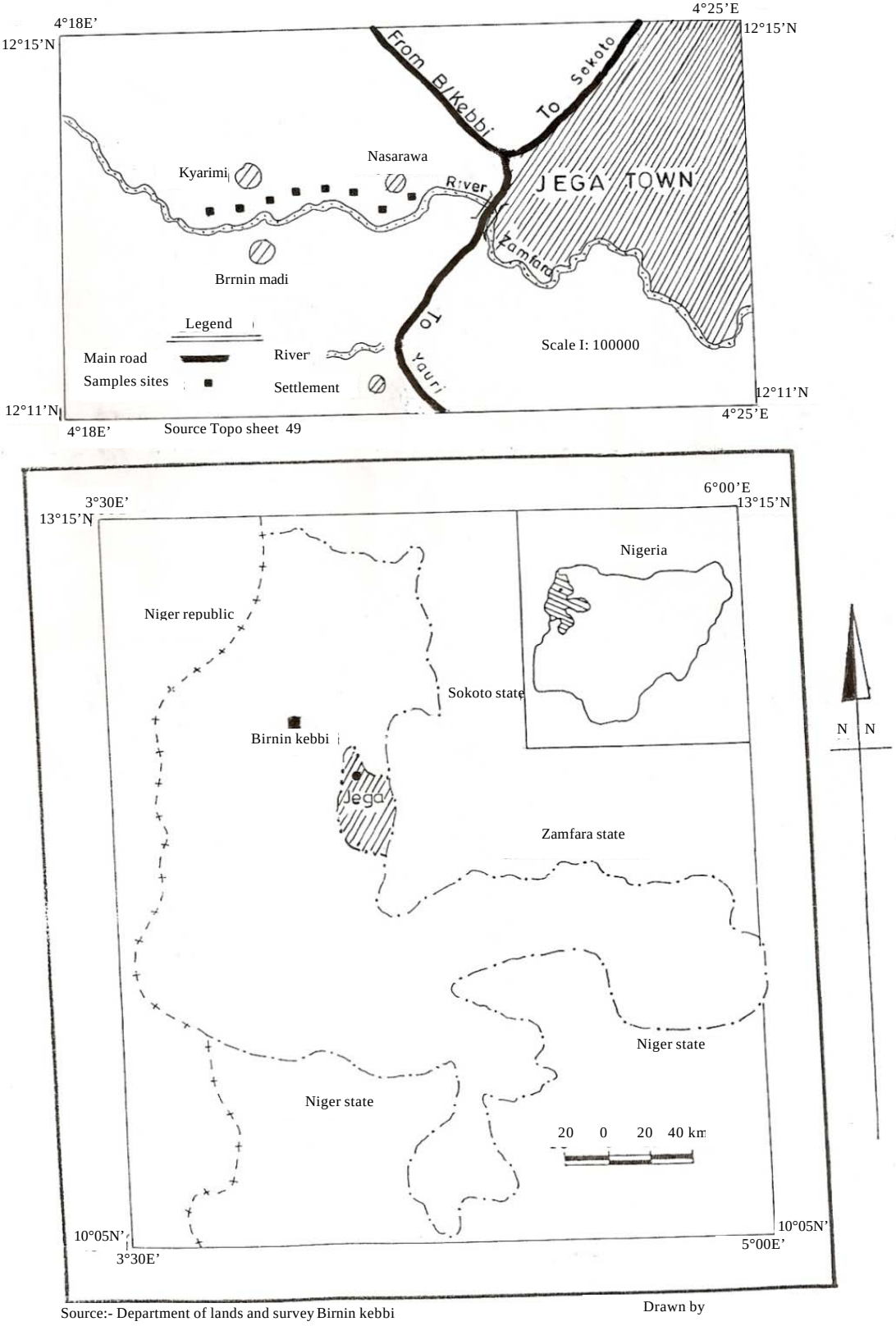

Fig. 1: Map showing study area and sampling sites

University, Zaria. The animals were housed in stainless steel cages and allowed to acclimatise to the laboratory conditions for two weeks. During the period, clean tap-water and feed (Bendel feed poultry growers mash) were supplied to the animals *ad libitum*. The rats were divided into two groups (five rats/group) based on balanced weight. Group 1 serves as control and fed with 100% poultry growers mash while group 2 were fed with 25% poultry growers mash and 75% *M. corchorifolia* pulverised leaves.

All the animals were weighed before the beginning and after the experimental period which lasted for 21 days. During the feeding trial, water was supplied *ad libitum*. During the feeding trial feed intake was calculated from the refusal and offered feed.

Collection of blood and organs samples: At the end of the 21st day, feed and water were withdrawn from the animal over night and weighed. The animals were anaesthetised in a container saturated with chloroform vapour, slaughtered and blood collected into labelled bottles coated with Ethylene Diamine Tetraacetic Acid (EDTA) as anti-coagulants. Also another sample of the blood was placed in labelled bottle without the anti-coagulant. The former and latter were used for haematological and biochemical assays respectively. The Liver and kidney of the scarified animals were fixed in 4% formalin-saline for histological examinations.

Biochemical analyses: The blood collected for biochemical assay was centrifuged at 2000 rpm for 10 min and serum decanted into clean 5 cm^3 sample bottle and kept at -20°C until analysis. The sera were used to analyse serum total protein, albumin aspartate aminotransferase activity, alanine aminotransferase activity, alkaline phosphatase activity, creatinine, bilirubin, urea, uric acid, glucose and electrolytes.

Serum total protein (Biuret method), albumin (Bromocresol Green (BCG) method), creatinine, glucose, bilirubin (total and direct), urea, uric acid (Phosphotungstate Method), aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities were determined by assay kits which were obtained from Randox laboratories Ltd., UK following manufactures instructions. The globulin concentration was obtained by subtracting albumin from the total protein. Serum Na^+ and K^+ were measured using flame emission spectrophotometer as describe by Cheesbrough (1991) while chloride was quantify using mercurimetric method reported by Schale and Schales (1941).

Haematological analyses: Anti-coagulanted blood was used to evaluate haemotological indices. Parked Cell Volume (PCV), Red Blood Cell count (RBC), white Blood Cell count (WBC) and platelets counts and haemoglobin (Hb) were determined using Wintrobe's micro-haematocrit, improved Newbauer haemocytometer and cyano-methaemoglobin method, respectively, as described by Lamb (1981), Lentowski and Ciesla (2007) and Ciesla (2007). The erythrocytic indices; Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were computed according to Lentowski and Ciesla (2007). Serum AST, ALT and ALP were determined by Reitman and Frankel (1957).

Histopathological tests: Samples of the liver and kidney from each animal were fixed in buffered 4% formalin-saline. After dehydration and embedding in paraffin wax, 5 μm sections were cut and stained with Myer's hematoxylin and eosin for examination under the light microscope for any changes in the tissues due to the consumption of *Melocia corchorifolia* leaves.

RESULTS AND DISCUSSION

Growth performance of rats fed wild leafy vegetables: The results of growth performance on rats fed with *Melocia corchorifolia* leaves was presented in Table 1. From the results, the rats fed with *M. corchorifolia* leaves experience decrease in body weight compared to the control group. Base on the study conducted by Umar *et al.* (2007), the leaves met all the nutritional requirements of the animals in terms of crude protein and fibre levels. However it might be right to assume palatability and antinutritional factors as the main causes of lower feed intake and negative weight gain which led to mortality of some rats. For instance *M. corchorifolia* leaves had high tannin content, which might hinder protein bioavailability (data unpublished). Palatability might also be responsible for the lower feed intake by the rats as also observed by James *et al.* (2009).

Haematological studies: The results of the present study as shown in Table 2 indicates that the erythrocyte count (RBC) was not significantly ($p>0.05$) different between control and sample treatment but above the normal range of $6.76\text{-}9.75\times10^{12}$ L^{-1} set for rats (The Rat Fan Club, 2010). Furthermore, the MCV, MCH and MCHC show no significant differences ($p>0.05$) between control and treatment. This showed that the plant species analysed may not cause anaemia. Haemoglobin and PCV in the treatment were all within the respective rats' normal range of 11.5-16.1 g L^{-1} and

Table 1: Body weight changes of rats fed with *M. corchorifolia* leaves

	Treatment	
Parameter	Control	*M.corchorifolia*
Initial weight (g)	142.10	140
Final weight (g)	152.90	94.82
Gain/loss in weight (g)	10.80	-45.18
ADG (g day^{-1})	0.51	-2.15
Feed intake (g day^{-1})	13.33	8.09
Feed: weight gain $lost^{-1}$	25.92	-3.76
Mortality	0/5.00	5-Feb.

ADG: Average daily growth

Table 2: Haematological Indices of Albino Rat Fed with *Melocia corchorifolia* leaves

	Treatment	
Parameter	Control	*M.corchorifolia*
WBC (109 dL)	12.75±0.72	11.10±0.10
RBS (1012 dL)	6.51±0.61	7.38±0.03
Haemoglobin (g L^{-1})	12.08±0.28	12.45±0.05
PCV (%)	44.75±0.85	44.50±0.50
MCV (fL)	68.78±1.75	60.34±0.47
MCH (pg)	18.56±0.57	16.88±0.13
MCHC (g dL^{-1})	26.98±0.34	27.98±0.43
Platelet (109 dL)	417.25±27.42	428.00±2.00
Lymphocyte (%)	80.50±3.59	79.00±1.00
Neutophils (%)	14.25±4.01	21.00±1.00
Monocytes (%)	4.50±0.87	2.00±0.00
Eosinophils (%)	0.75±0.48	ND
Basophils (%)	ND	ND

Values with asterisk (*) within the same row are significantly different at $p<0.05$, WBS: White blood cells, RBC: Red blood cells, PCV: Packed cell volume, MCV: Mean cell volume, MCH: Mean cell haemoglobin, ND: Not detected, MCHC: Mean cell haemoglobin conc

Table 3: Liver function parameters of albino rat fed with *Melocia corchorifolia* leaves

	Treatment	
Parameters	Control	Sample
Total protein (mg L^{-1})	7.54±0.35	6.63±0.130
Albumin (mg L^{-1})	3.28±0.20	2.46±0.040*
Globulin (mg L^{-1})	4.27±0.42	4.16±0.160
Total Bilirubin (mg dL^{-1})	0.38±0.05	0.44±0.000
Direct Bilirubin (mg dL^{-1})	0.22±0.07	0.31±0.0.02
ALT (IU L^{-1})	3.60±0.26	7.81±0.010*
AST (IU L^{-1})	14.50±0.54	43.55±0.050*
ALP (IU L^{-1})	106.26±1.78	165.65±0.050*
Glucose (mmole L^{-1})	5.12±0.21	3.20±0.100

Values with asterisk (*) within the same row are significantly different at $p<0.05$, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkalinephosphatase, IU L^{-1}: International unit

37.6-50.6% (The Rat Fan Club, 2010). White Blood Cell (WBC) count and white cells deferential count are index of cellular immunity. In this study the WBC was within the rats, normal range of 6.6-12.6×10^9 L^{-1} (The Rat Fan Club, 2010). This indicates that the feeds do not affect the body immune system. However, Saidu (2005) argued that WBC without deferential count is deceptive because granulocytes (neutrophils, eosinophils and basophils) which are phagocytic in nature are produced resulting in an increase in WBC. Eosinophils and basophils, which are Not Detected (ND) in the treatment are both within the normal range of 0-6 and 0-1%, respectively (The Rat Fan Club, 2010). High concentration of monocyte and lymphocyte are associated with chronic inflammatory disorders and chronic leukaemia respectively (Saidu, 2005). The lymphocyte and monocyte concentrations in the sample treatment are within the normal range of 65-85 and 0-6%, respectively (The Rat Fan Club, 2010). This is an indication that rats fed with the leaves showed no sign of microbial infection which may lead to disorder like leukaemia. Platelets play a significant role in blood clotting. In this study, no significant differences ($p>0.05$) were observed between control and rats fed with plant treatment.

Liver function test: Liver plays an important role in the body considering its function in detoxification of metabolic processes (Helal *et al*., 2008). The detoxification process disturbs the integrity of cell membrane, which may damage/ impair the liver function. Thus, change in the concentrations of total proteins, bilirubin, albumin and enzymes (AST, ALP and ALP) in the serum may indicate the state of the liver and the type of damage (Ashafa *et al*., 2009).

Total protein level is associated with evaluation of hydration status or possible haemorrhage and is a marker for acute and chronic active inflammation (Boonprong *et al*., 2007). From the results (Table 3), the concentration of total protein show no significant differences ($p>0.05$) between control and sample treatment and is within rats, normal values of 5.6-7.6 g dL^{-1} (The Rat Fan Club, 2010). Albumin concentration was significantly higher in rats fed with sample treatment compared to the control while globulin was not significant differet ($p>0.05$) between control and sample treatment. Furthermore, both the control and the samples had albumin content below the normal values of 3.8-4.8 g L^{-1} for rats (The Rat Fan Club, 2010). Albumin which is synthesised by the liver is the major form of protein present in the blood, and its low concentration is a marker of liver damage (Oboh *et al*., 2005). Thus, the result can be indicative of chronic liver damage.

Oboh *et al.* (2005) also reported that malnutrition could also cause hypoabunimemia, with no associated liver disease. In this analysis, generally, the latter reason was supported by histological studies and low average daily feed intake (Table 1).

Bilirubin concentration is another marker of liver dysfunction as elevation of total bilirubin concentration causes haemolysis or red blood cells in the liver (Oboh and Akindahunsi, 2005; Anosike *et al.*, 2008). In this study, there were no significant differences ($p<0.05$) between rats fed with control and those fed *M. corchorifolia* leaves. Furthermore, the treatment was also observed to have total bilirubin level within the normal values (0.2-0.55 mg dL^{-1}) for rats. This suggests that the secretory function of the liver was not impaired i.e., the feeds did not cause haemolysis of red blood cells.

There are many enzymes such as alanine amino transferase (ALT), Aspartate amino transferase (AST) and alkaline phosphatase (ALP) that are found in the serum which do not originate from the extracellular fluid. Conversely, during tissue damage, some of these biomolecules find their way into the serum through the disruption of cell membranes (Ashafa *et al.*, 2009). Serum enzyme measurement therefore, provides a valuable tool in toxicity studies. The significant increase ($p<0.05$) in the enzyme activity in the treatment compared to control implied cellular damage to the plasma membrane of the rats' organs, which is an indication that the sample was not completely safe in raw form perhaps unless they are processed. Also reduction in the serum enzymes may imply inhibitions at the cellular level by the component of the extract. In this study, even though there is significant increase in ALT and AST activities, which are signs associated with the treatment phytotoxin, the ALT level is below the rats' normal range of 17.5-30.2 and 45.7-80.8 U L^{-1}, respectively (The Rat Fan Club, 2010). Oboh and Akindahunsi (2005) argued that ALT is regarded to be a more specific indicator of liver inflammation because hyper AST may be caused by other diseases of other organs such as heart and muscles. They further stated that, mild or moderate (100-300 U L^{-1}) elevations of ALT and AST are non-specific and may be caused by a wide range of liver diseases. ALP activity in the sample treatment is above the normal values (56.8-128 U L^{-1}) which signifies cellular damage to the plasma membrane of the rats' organs; vis-à-vis the sample is not completely safe (Ashafa *el al.*, 2009).

The serum glucose level may indicate an adequate energy supply to the animals. Excess glucose is converted to glycogen by the liver. High level of glucose is an indication of certain liver diseases. In this study the glucose level differ not significant ($p>0.05$) between control and sample treatment and is also within the normal values (2.78-7.50 mmol L^{-1}) (The Rat Fan Club, 2010).

Kidney function test: Renal function indices (Table 4) are usually required to assess the normal functioning of different parts of the nephrons (Ashafa *el al.*, 2009). In this respect, the serum level

Table 4: Kidney function parameters of albino rat fed with *Melocia corchorifolia* leaves

	Treatment	
Parameter	Control	*M.corchorifolia*
Creatinine (mg dL^{-1})	0.79±0.09	1.15±0.99
Urea (mmole L^{-1})	5.99±0.45	6.69±0.01
Uric acid (mg L^{-1})	1.84±0.25	1.79±0.24
K+ (mmole L^{-1})	5.08±0.34	5.65±0.15
Na+ (mmole L^{-1})	139.50±2.02	122.50±0.50
Cl- (mmole L^{-1})	90.63±0.39	101.14±0.16
HCO_3-(mmole L^{-1})	28.50±1.50	30.27±0.94*

*Values with asterisk (*) within the same row are significantly different at $p<0.05$

of creatinine, urea, uric acid and electrolytes are significant markers of renal dysfunction by the feed consumed (Ashafa *el al.*, 2009). The serum level of creatinine of the normal treatment was generally not significantly different ($p>0.05$) with sample treatment. The rats fed with sample treatment has higher creatinine level compared to the rats' normal values (0.2-0.8 mg dL^{-1}) (The Rat Fan Club, 2010). This is an indication that the samples contained phyto-compounds that may cause kidney related malfunctions.

Urea is a nutritional pointer connected to protein intake and is useful in assessing kidney function in conjunction with creatinine which originates from the non-enzymatic conversion of creatinine in muscle and is filtered by the kidney (Boonprong *et al.*, 2007). The sample serum level is not significantly different ($p>0.05$) compared to control which signified no renal malfunction. Furthermore, the urea level was within the normal range of 2.50-22.5 mmol L^{-1} (The Rat Fan Club, 2010). Similarly, the serum uric acid was not significantly different ($p>0.05$) compared to control which further affirm no renal malfunction associated with the sample.

Correct levels of electrolytes in the body are of great importance as the osmotic balance in the body. Sodium and potassium ions are the major extracellular and intracellular fluid regulating acid-base balance. Hypernatraemia even though rare, is caused by dehydration while hyponatraemia is due to chronic sodium nephropathy and starvation among other causes (Saidu, 2005). Hyperkalaemia is common in renal failure due to nephropathy or renal tubular acidosis, whereas hypokalaemia could be due to poor dietary pattern (Saidu, 2005). High level of blood bicarbonate ions concentration is associated with respiratory acidosis and metabolic alkalosis, while low concentration is linked to respiratory alkalosis and metabolic acidosis (Saidu, 2005). In this study, these ions show no significant differences ($p>0.05$) between control and sample treatment. This is an indication that the sample may not have significant effect on variation of body acid-base balance. This also suggests that the sample may not cause renal dysfunction (Hassan *et al.*, 2005c).

Histopathological studies: From the results of histopathological studies, the histological sections of the livers and kidneys of rats fed with *M. corchorifolia* leaves showed features consistent with inflammation and congestion, these are non specific and are likely to result from mild tissue reaction. This is an indication that the liver and kidney show no sign of hepatic necrosis and tubular necrosis respectively.

CONCLUSION

The investigations reported herein were aimed to reveal the possible toxic effects of *Melocia corchorifolia* leaves. The result indicated decrease in body weight in rats fed with the leaves compared to those fed with normal diet which was attributed to present of antinutritional factors and non-palatability of the feed. Haematological indices showed that the leaves can not cause anaemia. Some biochemical parameters indicated that the leaves may have effect on liver and kidney functions while others show otherwise. Histological examinations showed features consistent with inflammation and congestion, which are non specific hence no sign of hepatic and tubular necroses. The results of this study showed that *Melocia corchorifolia* leaves have a relatively low or no toxicity profilere.

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