ISSN: 1119-1104

COMPARATIVE MORPHO-PHYSIOLOGICAL ASSESSMENT OF ACETAMINOPHEN-INTOXICATED KIDNEYS OF RATS PRE-TREATED WITH ETHANOLIC EXTRACTS OF *CUSCUTA AUSTRALIS*

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(Received 10th September 2013; Accepted 23rd December 2013)

ABSTRACT
Acetaminophen (APAP) overuse during fever and pain management is a well recognized threat to kidney architecture and function. *Cuscutaaustralis* extract pretreatments may be helpful against this deleterious effect. This study investigates the comparative morpho-physiological assessment of the kidneys of acetaminophen-intoxicated rats pre-treated with ethanolicextracts of *Cuscutaaustralis*. Thirty-six rats were randomly divided into six groups: Group I servedas the control and received distilled water. Group II was orally intoxicated with 835mg/kg body wt. of acetaminophen on day 8. Groups III and IV were orally administered the seed of *C. australis extract* in doses of 125mg/kg and 250mg/kg respectively for 7 days and then intoxicated as in Group II on the 8th day. Groups V and VI received *C. australis* stem treatment of similar doses and duration as in seed counterpart and then intoxicated as in Group II on the 8th day. Kidney sections and blood samples were taken for histopathological and biochemical evaluations respectively. Group II rats showed severe distorted renal architecture, decreased proximal and distal tubular diameters and significantly elevated blood urea nitrogen and creatinine levels. The seed and stem of *Cuscutaaustralis*extracts markedly improved the renal histoarchitecture and the injury markers in a dose-dependentmanner with higher efficacy shown by the stem treated Groups V and VI. This data suggest that ethanol extraction of *Cuscutaaustralis*stem appear to protect the kidney structure and function from acetaminophenintoxication better than the seed counterpart.
Keywords: Acetaminophen,*Cuscutaaustralis*, kidney, histoarchitecture, renal injury markers, rat.

INTRODUCTION
Acetaminophen (N-acetyl-p-aminophenol, APAP), also called paracetamol is a readily available antipyretic analgesic drug of fame commonly used for curing fever and pains. However, an overuse of acetaminophen has been recognized to be toxic to both the liver and extrahepatic tissues such as the kidneys (Gu*et al.*, 2005). The liver plays significant role in the metabolism of APAP as approximately 63% of acetaminophen is metabolized via glucuronidation and 34% by sulfationwhich are phase II reactions occurring primarily in the liver. Only 1% of the drug metabolites are excreted via the kidney (Mazer and Perrone, 2008). At therapeutic doses, 5% of APAP is oxidized by the microsomal P-450 enzyme system to a reactive intermediate, N-acetyl-p-benzoquinone (NAPQI) which under normal conditions is readily detoxified by conjugation with glutathione (GSH). The mechanism of acetaminophen toxicity is

well described in the liver and do occur when there is sustained overuse of acetaminophen which enhanced the buildup of NAPQI that covalently bind to cellular macro molecules (proteins and DNA) to produce protein adduct that terminates in acute hepatic necrosis (Dahlin*et al*., 1984; Mittal *et al*., 2010).

The mechanism is however, less clearly understood in the kidney. Oxidative stress is incriminated to play a key role in the pathogenesis of APAP-induced renal damage, as evidenced by an elevation in the lipid peroxidation (Balantz, 1996; Jaya *et al*., 1993) and the depletion of intracellular glutathione (GSH) level (Li *et al*., 2003; Trumpher*et al*., 1992). APAP-induced renal insufficiency is consistently reported to include acute tubular necrosis, an increase in the plasma creatinine level and a decrease in the glomerular filtrationrate (Cobden *et al*., 1982; Blakely and McDonald, 1995).

Due to the ability of APAP in causing life-threatening renal damages, the antidote or treatment of APAP-induced nephrotoxicity has a toxicological significance. Although N-acetyl-cysteine, a GSH precursor, has been established to protect against APAP hepatotoxicity in humans (Engelhardt and Hopmma, 1996; Prescott, 1993), it is not protective against APAP-induced renal injury (Davenport and Finn, 1988; Blakely and McDonald, 1995). Previous studies have shown that antioxidants such as melatonin, vitamins E and C and phyto-oxidants from *Curcuminlonga* prevented APAP-induced hepatotoxicity and nephrotoxicity in mice and rats (Sener*et al*., 2003; Abraham, 2005; Cekmen*et al*., 2009).

*Cuscutaaustralis*is an annual creeping parasitic plant that wraps around other plants for sustenance and constitutes the major flora of the tropical East and West Africa, Japan and Australia (Maria, 1987).*C. australis*seed and stem are famous among the Southwestern inhabitant of Nigeria for the treatment of various health challenges (Burkhill, 1985). Its seed has been reported to predominantly contain both kaempferol and astragalin while the stem contains a high level of kaempferol (Ye *et al*., 2002). In a previous study, Folarin*et al.* (2014) reported the comparative hepatoprotective effects of *Cuscutaaustralis*against acetaminophen-intoxication in rats. However, there is no documentation on the structure and function of the kidneys of acetaminophen-intoxicated rats pre-treated with aqueous *C. australis*extracts; this study seeks to investigate the possibility.

MATERIALS AND METHODS

Chemicals

Acetaminophen (Panadol®) tablet was purchased from GlaxoSmithKline Consumer Plc.

Plant Material Collection and Verification

The seeds and stems of *Cuscutaaustralis*wereharvested from Agogo Ide compound, Abeokuta, Nigeria. The plant has earlier been verified in the Department of Botany, University of Ibadan, Nigeria (Ozegbe and Omirinde, 2012).

Plant Extraction Procedure

The extraction procedure as stated in previous work, Folarin*et al*. (2014), entailed the use of three hundred (300 g) grams of *Cuscutaaustralis* seed and stems each. These were dried at room temperature, pulverized into powderusing motorized mill and mixed with 300mL of ethanol with a Soxhlet extractor at 20^0C for 6hours. The resultant filtrate was concentrated using a rotary evaporator (Bibby Sterling®, Germany) to produce 18.34 g (6.11% w/w) residue weight of seed and 15.92 g (5.31% w/w) of stem.

Animal Protocol

Thirty six healthy male wistar rats (150 – 200g) obtained from the experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, were used for this study. The rats were housed in galvanized wire mesh cages, under hygienic conditions. They were freely ventilated and naturally illuminated. All animals were fed with pelletised growers mash and water *ad libitum*. All rats received humane care in accordance to the "Guide for the care and use of Lab Animals" (National Academic Press, Washington DC, USA, 1996).

EXPERIMENTAL DESIGN

Rats were randomly divided into six groups following the modified experimental procedure of Yen *et al*. (2007). Each group consists of six rats as follows:

Group I (normal control)**:** Rats in this group received distilled water orally for seven days and then intraperitoneally injected with 10ml/kg body weight isotonic 0.9% Nacl on the 8th day.

Group II (acetaminophen control): Rats in this group received sterile water orally for seven days and then intoxicated by intraperitoneal injection of 835mg/kg body weightacetaminophen on the 8th day as earlier described by Yen *et al*. (2007).

Group III: was orally administered 125 mg/kg body weightof *C. australis* seed extract for seven days, and then intoxicated with 835mg/kg acetaminophen on the 8th day.

Group IV: was orally given 250 mg/kg body weightof *C.australis* seed extract forsevendays, and then intoxicated with 835mg/kg acetaminophen on the 8th day.

Group V:was orally administered 125 mg/kgbody weightof *C. australis* stem extract for seven days, and then intoxicated with 835mg/kg on the 8th day.

Group VI: was treated orally with 250 mg/kgbody weightof *C. australis* stem extract forseven days, and then intoxicated with 835mg/kg acetaminophen on the 8th day. Acetaminophen tablet was dissolved in 40% polyethylene glycerol 400 for administration.

After 24 hours of acetaminophen intoxication, the gross weights of the rats were taken using a digital balance (Scout Pro. SPU 402, OHAUS Corporation, Pine Brook, New Jersey, USA). Blood samples were collected from the medial canthus of the eye into Lithium heparinised tubes, centrifuged at 3000rpm for 15 minutes using a centrifuge (CF-405 Gallenkhamp, England). The supernatant was collected for renal (blood urea nitrogen and creatinine) injury marker profiles. The rats were later humanely sacrificed by cervical dislocation and kidney tissues were harvested, weighed and processed for the evaluation of histological variations.

Histopathological preparations of the kidneys

The kidney tissues were fixed in 10% formalin, routinely processed and stained with haematoxylin and eosin for light microscopy at X100 and X400 magnifications. The treated and control slides were compared for the presence of lesions by a trained veterinary pathologist. Also, morphometric measurements (Glomerulus's area, Glomerulus's diameter, outer and inner proximal and distal tubular diameters) were taken by using Motic image plus 2.0 software.

Biochemical Assays

Blood urea nitrogen and creatinine levels were determined using commercially available kits obtained from Fortress diagnostics Ltd., United Kingdom.

Statistical Analysis
The data was statistically analysed using one-way analysis of variance (ANOVA) and Turkey was used for multiple comparisons at post hoc. The results were presented as group mean ± standard error of mean; while the level of significance was $p<0.05$.

RESULTS

Relative Weight of the Kidneys of Experimental Rats
The relative weight of the kidney (reno-somatic index) was not significantly ($p > 0.001$) different in APAP exclusively treated (Group II) rats (0.58 ± 0.02) compared to control (0.53 ± 0.02) and the *Cuscutaaustralis* pre-treated groups III (0.67 ± 0.07), IV (0.56 ± 0.02), V (0.66 ± 0.04) and VI (0.58 ± 0.03) (Fig. 1).

Histopathology of Renal Tissues
Acetaminophen intoxication induced renal tubular epithelial flattening/thinning and widespread interstitial congestion in group II rats (Fig. 2) and was of more severity in the latter compared to the *Cuscutaaustralis* pre-treated groups IV and V rats. The normal kidneys parenchyma (Fig. 2) was retained in the control, Groups III and VI rats.

Renal Histomorphometry
The Morphometric findings on the glomerulus and renal tubules (proximal and distal convoluted tubules) of acetaminophen-intoxicated rats pretreated with varied doses of *Cuscutaaustralis* extracts are illustrated in Tables I and II. The values of the glomerular diameter and area were not significantly different ($p>0.05$) between the normal control and the acetaminophen exposed groups (Table I).

Acetaminophen (APAP) exclusively-treated (group II) rats showed significantly reduced ($p<0.001$) inner and outer proximal convoluted tubular diameters compared to the control. However, *Cuscutaaustralis* pre-treated groups III, IV, V and VI showed significantly increased ($p<0.05$; $p<0.01$ respectively) diameter more remarkably by the stem pre-treated groups when compared to group II rats. Similarly, the diameters of the distal convoluted tubules reduced significantly in APAP exclusively-treated (group II) rats ($p<0.001$) when compared to the control. Conversely, the various doses of *Cuscutaaustralis* extracts significantly increased ($p<0.05$) the diameters of the distal convoluted tubules of groups III, IV, V and VI rats compared to the APAP-exclusively treated rats.

Biochemical Parameters
The effects of pre-treatment with *Cuscutaaustralis* extracts against acetaminophen induced variations on biochemical parameters (Figs. 3 and 4). The renal injury markers, blood urea nitrogen (BUN) and creatinine (CREAT) levels, significantly increased ($p < 0.001$) in APAP-induced Group II rats relative to control. Pre-treatment with low (125mg/kg) and high (250mg/kg) extract doses of *C.australis*seed significantly ($p < 0.01$ and $p < 0.05$, respectively) reduced the elevations. In the same vein, stem extract pair doses significantly ($p < 0.05$ and $p < 0.001$, respectively) ameliorated the increased values of BUN and CREAT in dose-dependent mode compared to exclusively APAP-intoxicated Group II rats' parameters (Fig. 3).

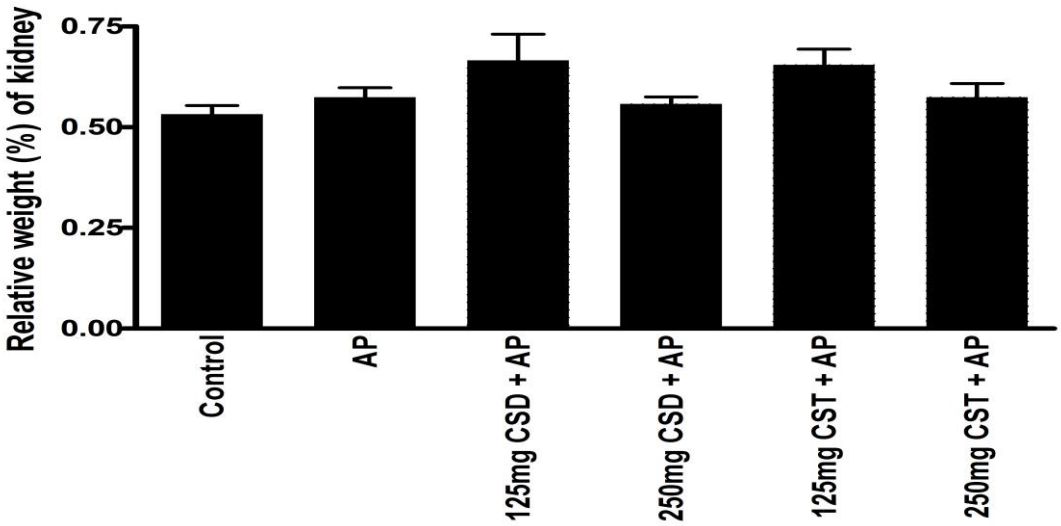

Fig. 1. Relative weight (%) of kidney of acetaminophen-intoxicated rats pre-treated with varied doses of *Cuscutaaustralis* extracts. AP/APAP (Acetaminophen) , CSD (*Cuscutaaustralis* seed), CST (*Cuscutaaustralis* stem)

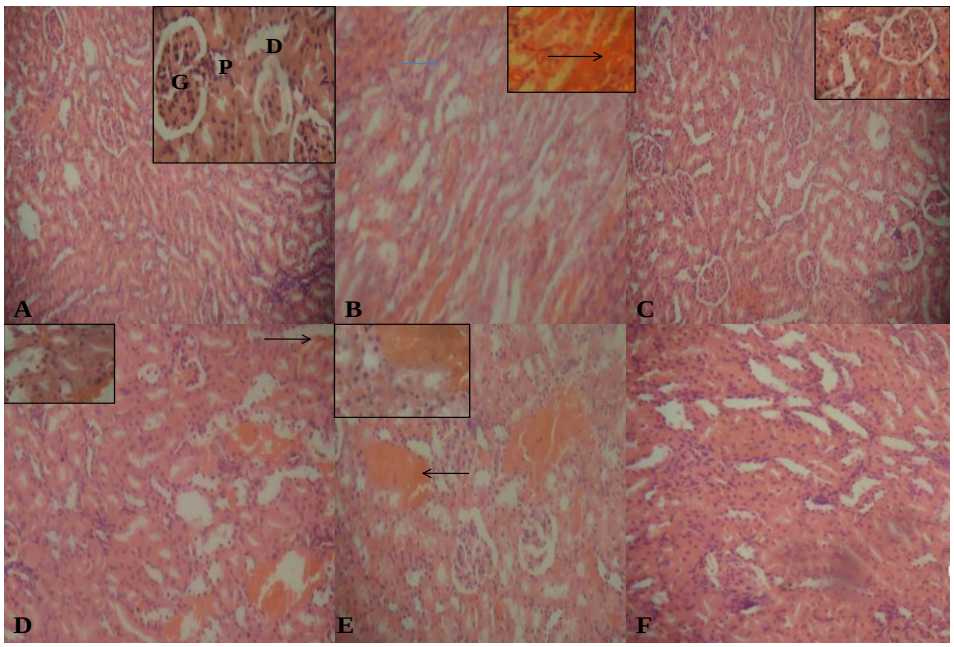

Fig. 2.Photomicrographs of the kidneys of rats.(A). Control; Renal cortex containing normal glomerulus (G), proximal (P) and distal (D) convoluted tubules.(B).Acetaminophen (AP/APAP); Severe thinning/flattening of the epithelium of tubules in the renal medulla (blue arrow), widespread interstial congestion (black arrow). (C).*Cuscutaaustralis*seed (CSD) 125mg + AP; No visible lesion.(D).*Cuscutaaustralis*seed (CSD) 250mg + AP; Focalinterstial congestion (black arrow). (E). *Cuscutaaustralis*stem (CST) 125mg + AP; Focalinterstial congestion (black arrow). (F).*Cuscutaaustralis*stem (CST) 250mg + AP; No visible lesion. Magnification (M): X100; Inset photomicrographs at M: X400; Stain: H&E.

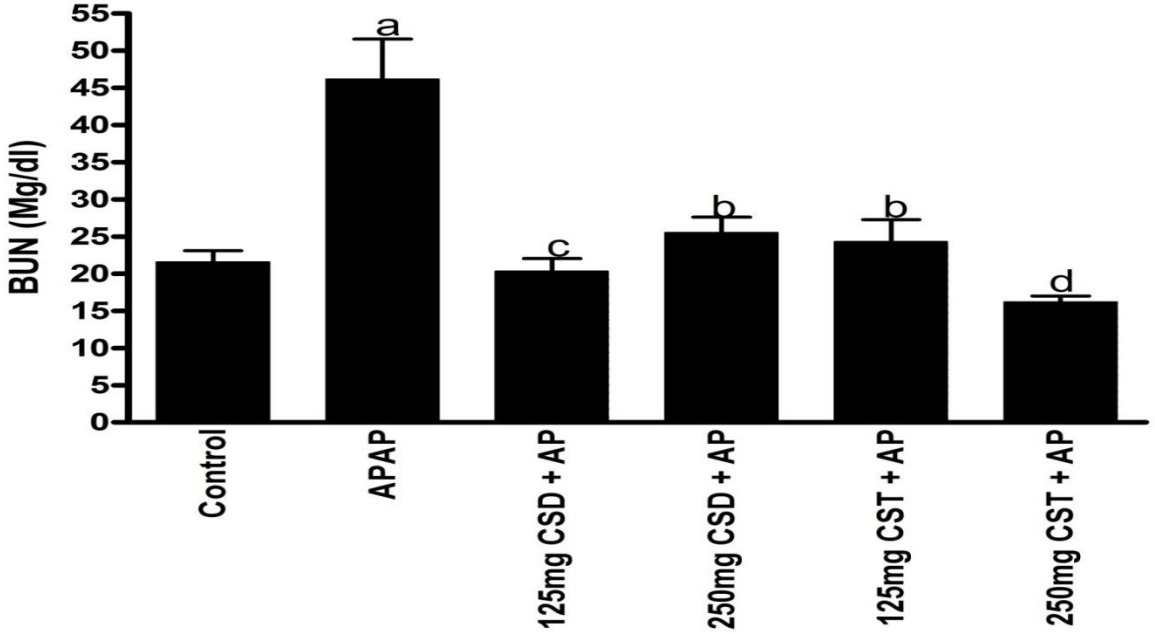

Fig. 3. The blood Urea Nitrogen level of acetaminophen-intoxicated rats pre-treated with varied doses of *Cuscutaaustralis* extracts. AP/APAP (Acetaminophen), CSD (*Cuscutaaustralis* seed), CST (*Cuscutaaustralis* stem). Values with different superscripts are significantly different; *p*-values: $^{a}p<0.001$-APAP compared with normal control; $^{b}p<0.05$, $^{c}p<0.01$, $^{d}p<0.001$- experimental groups compared with APAP.

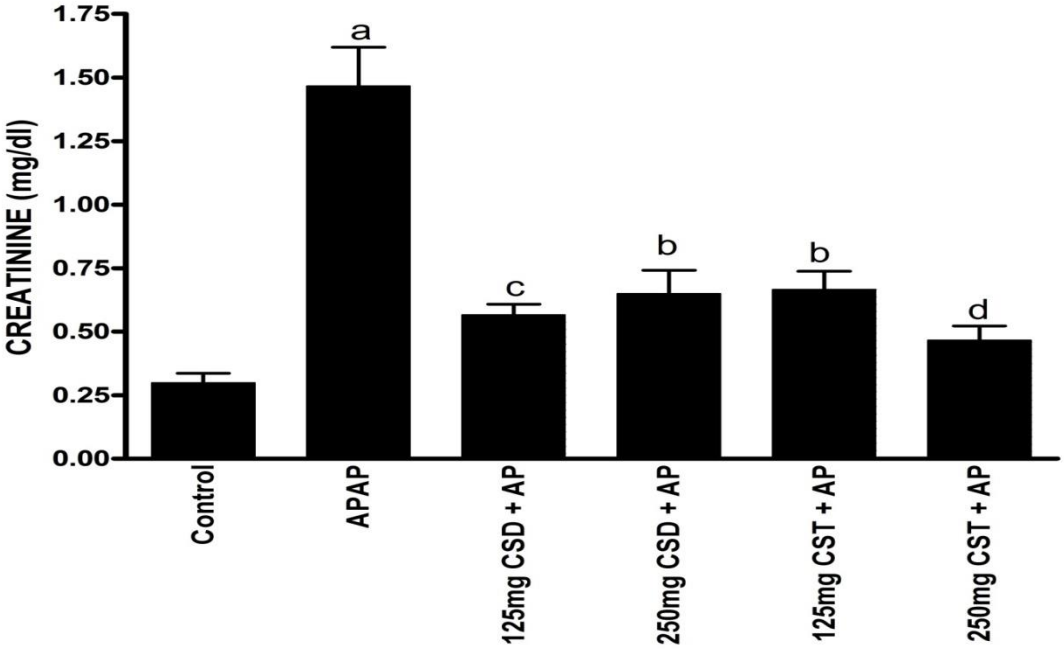

Fig. 4. Serum creatinine level of acetaminophen-intoxicated rats pretreated with varied doses of *Cuscutaaustralis*extracts.AP/APAP (Acetaminophen), CSD (*Cuscutaaustralis* seed), CST (*Cuscutaaustralis* stem). Values with different superscripts are significantly different; *p*-values: $^{a}p<0.001$-APAP compared with normal control; $^{b}p<0.05$, $^{c}p<0.01$, $^{d}p<0.001$- experimental groups compared with APAP.

Table I. Glomerular morphometrics (area and diameter) of acetaminophen-intoxicated rats pre-treated with varied doses of *Cuscutaaustralis* extracts

	Glomerulus Diameter (μm)	Glomerulus Area $(\mu m)^2$
Control	48.43 ±0.837	1781.820 ± 50.91
AP	46.09 ± 3.894	1374.523 ± 359.42
125mg CSD + AP	40.46 ± 8.89	1577.525 ± 156.17
250mg CSD + AP	49.34 ± 0.48	1391.415 ± 351.41
125mg CST + AP	41.22 ± 7.961	1459.827 ± 170.01
250mg CST + AP	48.92 ± 0.614	1695.410 ± 162.29

AP/APAP (Acetaminophen), CSD (*Cuscutaaustralis* seed), CST (*Cuscutaaustralis* stem)

Table II. Renal tubular morphometrics of acetaminophen-intoxicated rats pre-treated with varied doses of *Cuscutaaustralis* extracts

	Inner Proximal Tubule Diameter(μm)	Outer Proximal Tubule Diameter (μm)	Inner distal Tubule Diameter (μm)	Outer distal Tubule Diameter (μm
Control	35.19 ± 1.74	55.50 ± 1.68	13.32 ± 0.17	42.14 ± 1.55
APAP	17.05 ± 1.94^a	33.84 ± 1.99^a	7.296 ± 0.85^a	27.03 ± 0.95^a
125mg CSD + AP	32.80 ± 3.25^c	48.29 ± 4.66^c	10.82 ± 1.63^b	31.37 ± 2.36^b
250mg CSD + AP	21.27 ± 4.82^b	44.62 ± 1.06^b	9.820 ± 2.76^b	29.07 ± 3.20^b
125mg CST + AP	29.74 ± 5.01^b	51.23 ± 3.81^c	10.58 ± 1.08^b	32.37 ± 1.33^b
250mg CST + AP	33.34 ± 2.54^c	53.75 ± 2.62^c	11.55 ± 1.50^b	32.31 ± 1.88^b

AP/APAP (Acetaminophen), CSD (*Cuscutaaustralis* seed), CST (*Cuscutaaustralis* stem). Values with different superscripts are significantly different; p-values: $^a p<0.001$-APAP compared with normal; $p<0.05$, $p<0.001$-experimental groups compared with APAP.

DISCUSSION

This work demonstrated that acetaminophen (APAP) intoxication in Wistar rats could precipitate renal damage. This was evidenced by altered renal histomorphometry and deranged renal function markers. Hence, the observed renal toxicity is similar to previously documented reports on acetaminophen overdose (Mazer and Perrone, 2008; Cekmen*et al*., 2009;Pathan*et al*., 2013; Sabah *et al*., 2014).

The various histopathological lesions observed in the kidney architecture of the acetaminophen-intoxicated rats more importantly evident in the renal tubules of the exclusively exposed group II rats, are consistent with previous documentation on renal damage by acetaminophen intoxication (Kleinman*et al*., 1980; Cobden *et al*., 1982; Bjorck*et al*., 1988; Blakely and McDonald, 1995; Blantz, 1996). Similarly, the kidney morphometric findings of renal tubular diameters and a stable glomerular diameters and area of acetaminophen exposed rats corroborate the histological results from this study. Although, the lesion precipitated by acetaminophen intoxication is expected to influence the reno-somatic index (kidney mass as percent of body mass) of the exposed groups particularly the APAP exclusively intoxicated group II that do not have prior pretreatment with *C. australis*, however, the index remained. This could be suggested to be due to the short duration of this study; as the duration is

lengthened, there could be high tendency of falling reno-somatic index with kidney damage.

Remarkably, *Cuscutaaustralis* pretreatments ameliorated the extent of acetaminophen intoxication in this study by improving renal histo-architecture and tubular morphometric parameters particularly in groups (III, V and VI) that received the low and high doses of the seed andstem extracts respectively. These observed renalimprovement offered by these extracts seem to be dose dependentrelatedanda better dose-dependent response was shown by the stem extract pairs. Thisobservationagrees with the dose-response pattern earlier reported for *C. australis* effect on acetaminophen intoxicated liver cells (Folarin*et al*., 2014). The efficacy displayed by *C. australis* extracts lend credence to its anti-oxidative components (astragalin and kaempferol) that has earlier been reported in a similar study by Folarin*et al*. (2014) to have protected liver cells from acetaminophen overload.

The blood urea nitrogen and creatinine levels which are indicators of renal dysfunction (Adejuwon*et al*., 2014) markedly increased in the acetaminophen intoxicated rats probably due to oxidative damage to renal tissues. The latter is occasioned by increased level of highly reactive N-acetyl-p-benzoquinamine (NAPQI) in cytochrome P450 which is deacetylated to p-aminophenol and eventually converted to a free radical. The generated radical binds cellular proteins in convoluted tubules more particularly the proximal convoluted tubules which are primarily concerned with absorption and excretion of glomerulus filtered substances. These findings corroborate the typical elevated renal injury markers previously documented in APAP-intoxication (Cekmen*et al*., 2009;Pathan*et al*., 2013; Sabah *et al*., 2014).

It is striking to note that *C. australis*pre-treatments, most especially the stem extract, ameliorated the deranged renal function markers in dose-dependent manner by improving the profiles of the markers. The observed improvements could be ascribed to phyto-antioxidants components (astragalin and kaempferol) of *C. australis*and particularly the large concentration of kaempferol in the stem of *C. australis* (Ye *et al*., 2002).

The observed protective role of *C. australis* on the kidney structure and function in this study is similar to previous reportson*Curcuminlonga* Linn (Cekmen*et al*., 2009), *Maytenusemarginata*(Pathan*et al*., 2013) and *Eucalyptus globules* (Dhibi*et al*., 2014)extracts in a rat model of acetaminophen-inducednephrotoxicity. This is the first report on the protective effects of the stem and seed of *C. australis* on the kidney structure and function in Wistar rats and provides a new approach into the traditional use of this parasitic plant as a supplement for the management of renal diseases.

Conflict of Interests

The authors declare that there is no conflict of interests concerning the publication of this paper

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