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Effects of n-butanol fraction of *Gongronema latifolium* leave extract on some kidney function and histological parameters in CCl_4-induced oxidative damage in Wistar albino rats.

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ABSTRACT

Effects of n-butanol fraction of *Gongronema latifolium* leave extract on some kidney function and histological parameters in (CCl_4) carbon tetrachloride-induced oxidative damage in Wistar albino rats were assessed. Fifty-four (54) Wistar albino rats were divided into treatment and LD_{50} groups. The treatment group was further divided into seven groups of 6 animals each by the randomized random design method, each were allowed food and water ad libidium. Group A (normal control) was given feed and water, Group B (vehicle control) was injected with olive oil intraperitoneally, while the rest groups (C, D, E, F and G) were injected intraperitoneally with a single dose of CCl_4 (148 mg/kg) at 1:1 (v/v) solution in olive oil and all the animals were fasted for 36 hours. This was repeated once every week for a period of four (4) weeks. At the end of 28 days of treatment, there was significant ($p<0.05$) reduction in weight change of CCl_4-induced control rats when compared with the normal control and induced treated groups. Kidney function studies showed that there was significant ($p<0.05$) increase in creatinine and urea levels of CCl_4-induced control group when compared with the normal control and induced treated groups but there was no significant ($p>0.05$) difference between the normal control and induced treated groups. Also, the kidney homogenate revealed significant ($p<0.05$) decrease in superoxide dismutase, catalase and glutathione peroxidase activities in CCl_4-induced control rats when compared with the normal control rats but there was no significant ($p>0.05$) difference between the normal control and induced treated groups. However, malondialdehyde concentration was significantly ($p<0.05$) higher in CCl_4-induced control rats when compared with the normal control and induced treated rats. These findings suggested that n-butanol fraction of methanolic leave extract of *G. latifolium* may have anti-nephrotoxic and antioxidative effects against CCl_4-induced kidney damage in rats.

Keywords: ***Gongronema latifolium;*** **Antioxidant; CCl_4; n-butanol; Kidney.**

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INTRODUCTION

Gongronema latifolium (Asclepiadaceae), is a perennial climber forest leafy vegetable with woody hollow glaborous stems below and characterized by greenish yellow flowers (Okafor, 1989). It is widespread in tropical Africa such as Senegal, Chad and DR Congo as well as grows in the forest of south eastern and western Nigeria where it is widely used for medicinal and nutritional purposes (Ugochukwu *et al.*, 2003). *G. latifolium* occurs in rainforest, deciduous and secondary forests, and also in mangrove and disturbed roadside forest, from sea-level up to 900 m altitude. In Nigeria, information available from the indigenous traditional healers claimed that a decoction of the chopped (Ajibola and Satake, 1992) leaves of *G. latifolium* has been used in the production of several herbal products which are taken orally (Okafor, 1989) for the treatment of stomach upsets and pains, dysentery, malaria, typhoid fever, worm and cough (Akpan, 2004). Asthma patients chew fresh leaves to relieve wheezing (Okafor, 1989) and a decoction of the roots, combined with other plant species, is taken to treat sickle cell anaemia. A maceration of the leaves in alcohol is taken to treat bilharzia, viral hepatitis and as a general antimicrobial agent (Okigbo *et al.*, 2009). It is also taken as a tonic to treat loss of appetite (Akpan, 2004). Previous studies have revealed that other plants with polyphenols exhibit clear anti-nephrotoxic properties (Okafor, 1989), and that flavonoids could protect the kidney against oxidative injury induced by CCl_4 *in vivo* (Akpan, 2004). Although many other plants have been reported to possess anti-hepatotoxic properties, the scientific authentication of most of them such as *G. latifolium* which is used traditionally to treat several diseases is unavailable (Ajibola and Satake, 1992).The qualitative phytochemicals screening of the methanolic leave extract of *G. latifolium* revealed the presence of glycosides, alkaloids, saponin, flavonoids, tannins, and the absence of free anthraquinone. The quantitative analysis of phytochemical constituents of *G. latifolium* leaves is presented in Table 1. The crude extract showed high tannin content followed by glycosides, alkaloids and saponin. The results in Table 2 also showed that the n-butanol fraction has higher flavonoids, polyphenols and ascorbic acid content than the ethylacetate fraction. The aim of this work is to provide some scientific support for the health benefit of *G. latifolium*. To achieve this, studies were carried out to investigate the phytochemical constituents of *G. latifolium* and to evaluate the anti-nephrotoxic activities of n-butanol fraction of methanolic leave extract of *G. latifolium* against oxidative damage induced by CCl_4 in Wistar albino rats.

MATERIALS AND METHODS

Chemicals/Reagents

All assays kits were from Randox Laboratories Ltd. Ardmore, Co. Antrm UK. Chemicals and reagents used were purchased from Sigma Chemical Company St. Louis U.S.A. and chemicals used were of analytical grade. Folin ciocalteu phenol reagent, gallic acid, carbon tetrachloride (Sigma-Aldrich), distilled water, normal saline.

Plant Material and Extraction

Fresh leaves (blend) of *G. latifolium* were obtained from a homestead garden at Isuofia, Aguata L.G.A., Anambra State, Nigeria in the month of February 2013 and authenticated at the herbarium unit by Gallah U.J. in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria where a voucher specimen was deposited. The collected plants were rinsed in clean water and air dried at room temperature for two weeks. The dried leaves were pulverized into powder using Thomas-Wiley laboratory mill (model 4) manufactured by Arthur H. Thomas Company, Philadelphia, PA., U.S.A. before being extracted. A portion of five hundred grams (500 g) of the pulverized plant leaves was suspended in 2.5 L of methanol for 48 hours in large amber bottles with intermittent shaking. At the end of the extraction, the crude methanol extract was filtered using Whatmann No. 1 filter paper (1mm mesh size) and then concentrated for 24 hours over a water bath maintained at 45°C until greenish black residues were obtained. Certain gram of the crude extract was then subjected to phytochemical analysis using standard procedures (Sofowora, 1993). Also, 51 g of the crude extract was reconstituted with 250 ml of methanol for further fractionation and the fractions were kept in sealed containers and refrigerated at 2-4ºC for further use. The percentage yield of both the crude methanol leaves extract and fractions were determined as a percentage of the weight (g) of the extract to the original weight (g) of the dried sample used.

Fractionation of crude extract

The crude extract of *G. latifolium* was subjected to liquid- liquid partition separation to separate the extract into different fractions. 250 ml of the reconstituted extract was placed in a separator funnel and 250 ml of n-hexane, ethylacetate and n-butanol solvents were added sequentially as a 1:1 (v/v) solution and rocked vigorously (Abbot and Andrews 1970). The sample was left standing for 30 minutes for each solvent on the separator funnel until a fine separation line appear clearly indicating the supernatant from the sediment before it was eluted sequentially. The process was repeated thrice in order to get adequate quantity for each fraction. The n-hexane, ethylacetate, n-butanol as well as the aqueous residue fractions were evaporated to dryness over a water bath maintained at 45°C for 24 hours to afford four fractions in (grams) respectively.

Preliminary Phytochemical Screening

Test for Glycosides was carried out according to the method of Trease and Evans, 1983.

Test for Anthraquinones derivatives was carried out according to the method of Trease and Evans, 1983.

Test for Saponins was carried out according to the method of Trease and Evans, 1983.

Test for Flavonoids was carried out according to the method of Trease and Evans, 1983.

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Test for Tannins was carried out according to the method of Trease and Evans, 1983.

Test for Alkaloids was carried out according to the method of Sofowora, 1982.

Quantitative Analysis of Phytochemicals

Determination of saponin was carried out according to the gravimetric method of AOAC, 1984.

Determination of total flavonoids was done using the method of Boham and Kocipal-Abyazan (1974).

Determination of tannin was done using the standard method described by AOAC (1980).

Determination of Glycosides was done using the standard method described by AOAC (1984).

Determination of total phenolic contents (TPC) using the Folin-Ciocalteu method adopted by Amin *et al.*, 2004 was used.

Ascorbic Acid Contents was determined using the method described by Barros *et al.* (2007).

Determination of Alkaloids was carried out using the procedure described by Harbone (1973) with slight modification by Edeoga *et al.* (2005).

Animals

A total of 54 apparently healthy Wistar albino rats of both sexes weighing between 100-150 g were obtained from the animal house, Department of Pharmacology, Ahmadu Bello University, Zaria, Kaduna State. The animals were separated into male and female in well aerated laboratory cages in the animal house, Department of Pharmacology, Ahmadu Bello University, Zaria, Kaduna State and were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment. They were fed daily with grower mash from Vital Feeds Company and water *ad libitum* during the stabilization period.

Acute Toxicity Study

The median lethal dose (LD_{50}) of n-butanol fraction was conducted in order to select a suitable dose for the evaluation of the effects of n-butanol fraction. This was done using the method described by Lorke (1983). In the initial phase, rats were divided into 3 groups of 3 rats each and were treated with 10mg, 100 mg and 1000 mg of n-butanol fraction per kg body weight orally. They were observed for 24 hours for signs of toxicity, including death. In the final phase, 3 rats were divided into 3 groups of one rat each, and were treated with n-butanol fraction based on the findings in the first phase. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e. the geometric mean of the consecutive doses with 0 and 100% survival rates were recorded.

Animal Grouping

A total of 54 Wistar albino rats were used. The rats were divided into carbon tetrachloride induced kidney damage group of 6 rats each and LD_{50} group.

Carbon tetrachloride induced group

Group A: Normal control Rats were given feed and water only. This served as the normal control group (NC)

Group B: Rats were treated with olive oil and served as vehicle control group (VC)

Group C: Rats were treated with 148mg/kg b.wt. carbon tetrachloride (CCl_4) in olive oil. This serves as the CCl_4-induced liver damage group (IC).

Group D: Rats were treated with 148 mg/kg b.wt. CCl_4 in olive oil + 100 mg/kg b.wt. Silymarin as standard drug (CCl_4+ Std)

Group E: Rats were treated with 148 mg/kg b.wt. CCl_4 in olive oil + 100 mg/kg b.wt. n-butanol fraction. (CCl_4+BF)

Group F: Rats were treated with 148 mg/kg b.wt. CCl_4 in olive oil + 150 mg/kg b.wt. n-butanol fraction. (CCl_4+BF)

Group G: Rats were treated with 148 mg/kg b.wt. CCl_4 in olive oil + 200 mg/kg b.wt. n-butanol fraction. (CCl_4+BF).

Induction of Kidney Damage

The kidney damage was induced by the administration of carbon tetrachloride (CCl_4). Rats were injected intraperitoneally with a single dose of CCl_4 (148 mg/kg body weight) as a 1:1 (v/v) solution in olive oil and were fasted for 36 hours before the administration of n- butanol fraction (Manoj and Aqued, 2003). This was done once a week for a period of four weeks. The administration of n- butanol fraction was done daily by oral intubation for the period of 28 days.

Collection and Preparation of Sera Samples

At the end of 28 days of treatment, the animals were sacrificed by decapitation using chloroform anaesthesia and blood samples were collected from the head wound in plain bottles (for biochemical parameters). The Blood samples collected in plain tubes were allowed to clot and the serum separated by centrifugation using Labofuge 300 centrifuge (Heraeus) at 3000 rpm for 10 minutes and the supernatant (serum) collected was subjected to biochemical screening.

Collection of Kidneys

Immediately after the blood was collected, the kidneys was quickly excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed (so as to calculate the relative weight) and kept on ice. Certain gram of the kidney was crushed in 50mM potassium phosphate buffer (pH 7.4) using mortar and pestle (homogenization) while the rest of the organs were placed in freshly prepared 10% formalin for histopathological studies. It was then centrifuged at 4000 rpm (2700 xg) for 15 minutes. Then the supernatant was collected using Pasteur pipette. The percentage change in organ weight of each of the animals was calculated as follows;

% change in weight $= \frac{\text{organ weight}}{\text{animal weight}} \times 100$

Heamatological Assay

Determination of Packed Cell Volume (PCV): The PCV is the volume of red blood cells (RBC) expressed as a fraction of the total volume of the blood. The microhaematocrit method was used (Cheesbrough, 2000).

Biochemical Studies

Determination of Serum Urea Concentration: This was assessed using the method described by Fawcett and Scout (1960).

Determination of Serum Creatinine Concentration: The colorimetric method was used to determined serum creatinine concentration according to Bartels and Bohmer (1973).

Estimation of Superoxide Dismutase (SOD) Activity: Superoxide dismutase activity was measured using the method described by Martin *et al*., 1987.

Estimation of Catalase Activity: Catalase activity was determined using the method described by Aebi and Bergmeyer (1983).

Estimation of Glutathione Peroxidase: Glutathione peroxidase assay was determined using the method adapted by Paglia and Valentine, 1967.

Estimation of Thiobarbituric Acid Reactive Substance (TBARS): Thiobarbituric acid reactive substance (TBARS) in the tissues was estimated in the form of (MDA) using the method described by Fraga *et al.,* (1988).

Histopathological Studies

A portion of the kidney of the animals was cut into two to three pieces and fixed in 10% formalin (Lillie, 1965). The paraffin sections were prepared and stained with haematoxylin and eosin. The thin sections of kidneys were made into permanent slides and examined under high (X250) resolution microscope with photographic facility and photomicrographs were taken.

STATISTICAL ANALYSIS

The data were analyzed by the analysis of variance (ANOVA) using SPSS program (version 17.0 SPSS Inc., Chicago, IL, USA). The differences between the various animal groups were compared using the Duncan Multiple Range Test. The results were expressed as mean ± standard error of mean (SEM). P value less than 0.05 was considered as significant ($P < 0.05$).

RESULTS

The Percentage Yield of Methanolic Leave Extract and Fractions of *G. latifolium*

The percentage yield (w/w) of the crude extract is (10.24%) and the various fractions have aqueous residue as the highest yield (45.80%), followed by n-butanol fraction (25.14%), ethylacetate fraction (10.70%) and n-hexane fraction has the lowest yield (6.66%).

Table 1: Quantitative Analysis of the Phytochemical Constituents (mg/g) of *G. latifolium*

Leave	Alkaloids (mg/g)	Saponins (mg/g)	Glycosides (mg/g)	Tannins (mg/g)
Crude	1.26	0.82	2.57	10.60

Table 2: Quantitative Analysis of the Phytochemical Constituents (mg/g) of fractions of *G. latifolium*

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	Fractions	Polyphenols (mg/g)	Flavonoids (mg/g)	Ascorbic acid (mg/g)
n- Butanol	4.53	5.15	2.24	
Ethylacetate	2.39	4.51	0.62	

Lethal Dosage (LD_{50}) determination for n-butanol fraction of *G. latifolium*

No death was recorded after the oral administration up to a dose of 5000 mg per kg body weight.

Effects of n-butanol fraction of *G. latifolium* on Packed Cell Volume

The effect of sub-chronic oral administration of n-butanol fraction of *G. latifolium* methanolic leaves extract and silymarin (Standard drug) at 100 mg/kg b.wt, 150 mg/kg b.wt and 200 mg/kg b.wt. on packed cell volume in CCl_4-induced kidney damage in albino rats for 28 days is shown in Figure 1. The result showed that the packed cell volume (PCV) level of induced control group was significantly ($P<0.05$) lowered than the PCV level of normal control group, but there was no significant ($P>0.05$) difference between the PCV level of the normal control animals and all the induced treated animals.

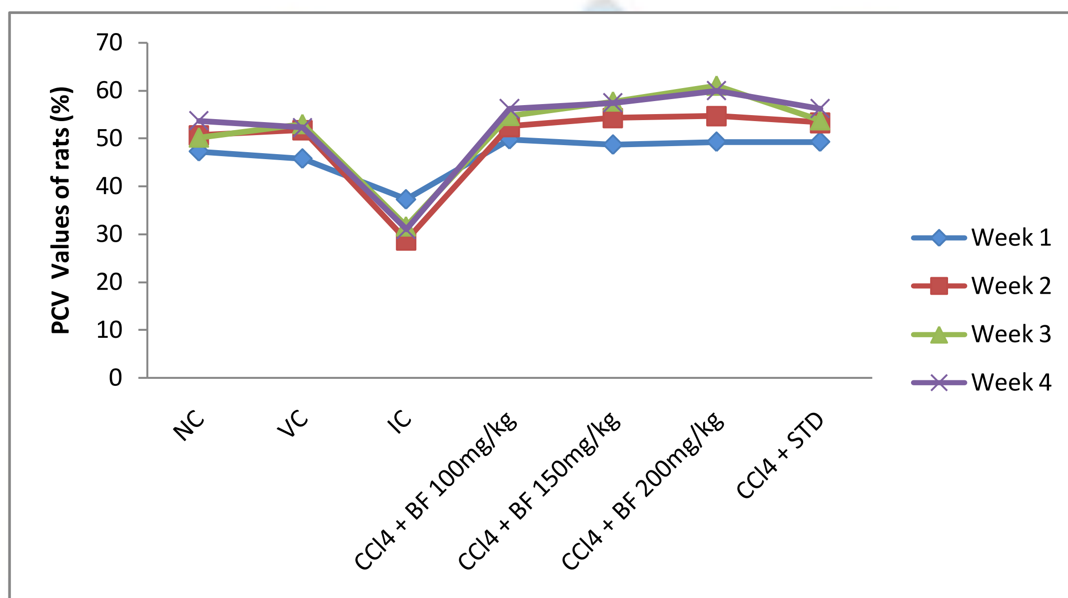

Figure 1: Mean changes in PCV values of CCl_4-Induced Kidney damage rats treated daily with oral administration of n-butanol fraction of *G. latifolium* and silymarin (STD).

Values are presented as mean with six replicates for each group.NC: Normal Control rat, VC: Vehicle control rats, CCl_4: Carbon tetrachloride, IC: CCl_4 Induced kidney damage control rats, CCl_4 + BF: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+150mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl_4 + Std: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin).

Effects of n-Butanol Fraction of *G. latifolium* on Body and Organ Weight Change.

Changes in body weight of rats induced kidney damage treated with n-butanol fraction of *G. latifolium* methanolic leaves extract and silymarin (Standard drug) for a period of 28 days is represented in Figure 2. The results showed no significant ($P>0.05$) difference in the body weight change of all the induced treated groups compared with the normal control group. However, the CCl_4 induced kidney damage control group shows a significant ($P<0.05$) decrease in body weight compared with the induced treated and normal control groups.

Changes in organ weight of rats induced kidney damage treated with n-butanol fraction of *G. latifolium* methanolic leaves extract and silymarin (Standard drug) for a period of 28 days is represented in Table 3. The result showed that there was no significant ($P>0.05$) difference between the percentage change in kidney weights of the entire induced treated group compared with the normal control rats. However, the induced control rats presents a significant ($P<0.05$) higher percentage change in kidney weights compared with the normal control rats.

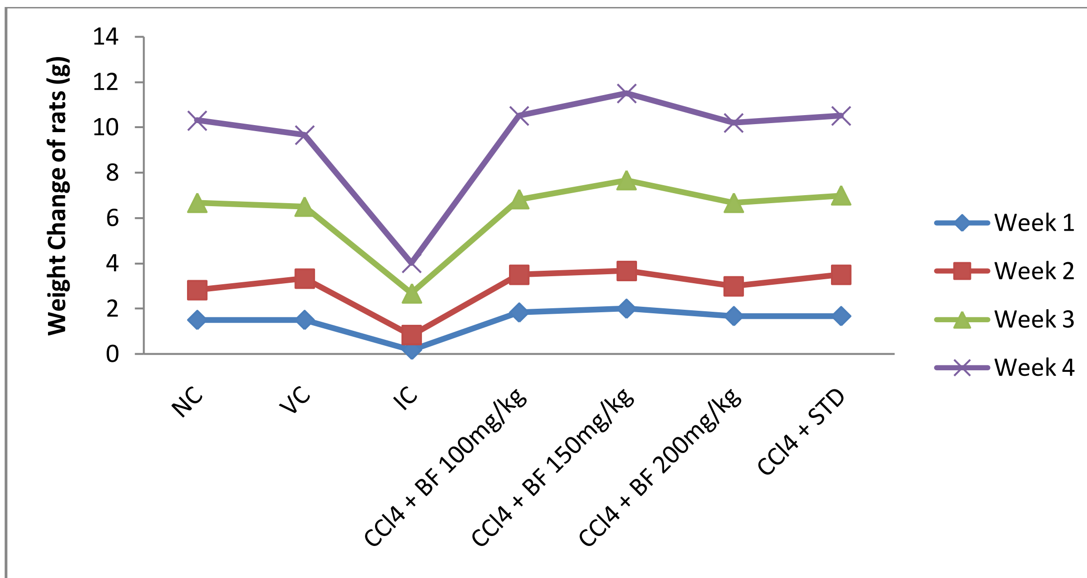

Figure 2: Mean Changes in body weights of CCl_4-Induced Kidney damage rats treated daily with oral administration of n-butanol fraction of *G. latifolium* and silymarin (STD)

Values are presented as mean with six replicates for each group.

NC: Normal Control rat, VC: Vehicle control rats, CCl_4: Carbon tetrachloride, IC: CCl_4 Induced kidney damage control rats, CCl_4 + BF: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+150mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl_4 + Std: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin).

Table 3: Mean Changes in Organ Weights of CCl_4-Induced Kidney Damage Rats Treated Daily with Oral Administration of Silymarin and n-Butanol Fraction of *G. latifolium*.

Groups (n=6)	% Change in Kidney Weight (g)
NC	$0.52{\pm}0.01^{a}$
VC	$0.58{\pm}0.02^{a}$
IC	$0.85{\pm}0.05^{b}$
CCl_4+ BF	$0.57{\pm}0.03^{a}$
CCl_4+ BF	$0.52{\pm}0.02^{a}$
CCl_4+ BF	$0.51{\pm}0.01^{a}$
CCl_4+ Std	$0.55{\pm}0.03^{a}$

Values are Means ± SEM. Values with different superscript down the columns are significantly different ($P<0.05$) NC: Normal Control rat, VC: Vehicle control rats, CCl_4: Carbon tetrachloride, IC: CCl_4 Induced kidney damage control rats, CCl_4 + BF: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+150mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl_4 + Std: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin).

Biochemical Studies

Effects of n-Butanol Fraction of *G. latifolium* on Kidney Function Parameters.

Creatinine concentrations in the serum of normal and CCl_4 induced kidney damage rats after the oral administration of n-butanol fraction of *G. latifolium* and silymarin for 28 days is represented in Figure 3. The results showed that the concentration of creatinine in the serum of CCl_4 induced not treated rats was significantly ($P<0.05$) higher when compare with the normal control rats. However, there was no significant ($P>0.05$) difference between the concentration of creatinine in all the induced treated groups when compared with the normal control group.

Figure 4 depicts the urea concentrations in the serum of normal and CCl_4 induced kidney damage rats after the oral administration of n-butanol fraction of *G. latifolium* and silymarin for 28 days. The results showed that the concentration of urea in the serum of CCl_4 induced not treated rats was significantly ($P<0.05$) higher when compared with normal control rats and all the induced treated groups. However, there was no significant ($P>0.05$) difference between the concentration of urea in the serum of all the induced treated groups when compared with the normal control group.

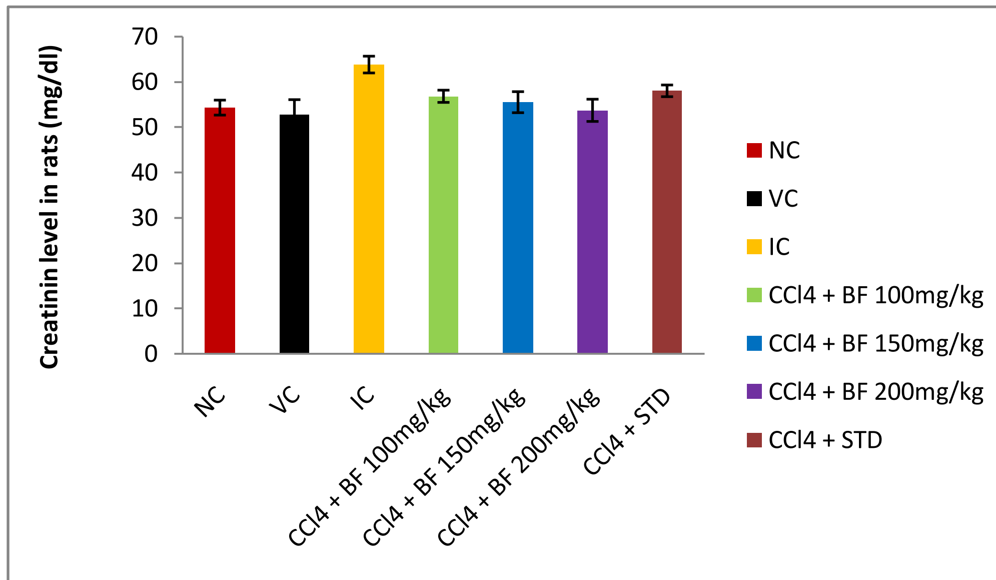

Figure 3: Effects of n-butanol fraction of *G. latifolium* on Serum Creatinine level of CCl_4-Induced Kidney Damage Rats.

Values are presented as Mean ±SEM for each Bar with six replicates for each group.

NC: Normal Control rat, VC: Vehicle control rats, CCl_4: Carbon tetrachloride, IC: CCl_4 Induced kidney damage control rats, CCl_4 + BF: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+150mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl_4 + Std: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin).

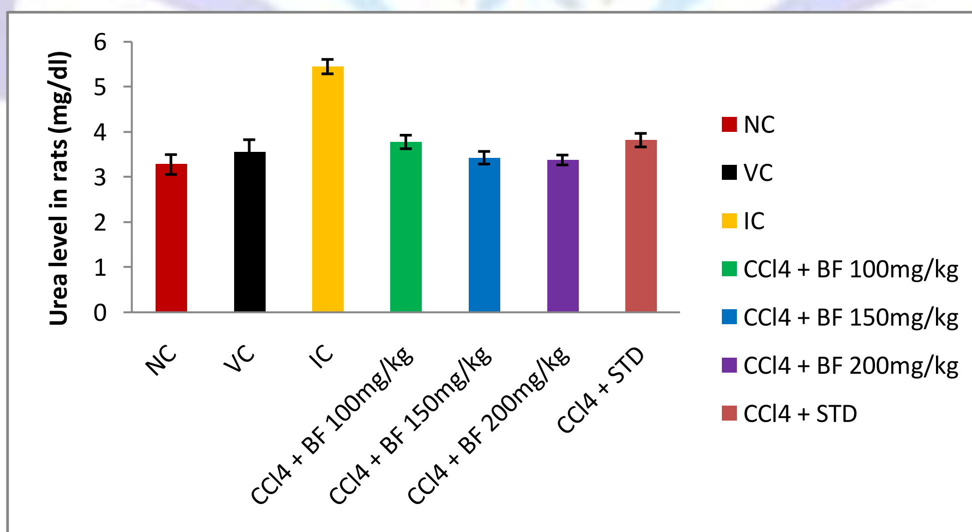

Figure 4: Effects of n-butanol fraction of *G. latifolium* on Serum Urea level of CCl_4-Induced Kidney Damage Rats.

Values are presented as Mean ±SEM for each Bar with six replicates for each group. NC: Normal Control rat, VC: Vehicle control rats, CCl_4: Carbon tetrachloride, IC: CCl_4 Induced kidney damage control rats, CCl_4 + BF: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+150mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl_4 + Std: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin).

In vivo Antioxidant Studies.

Effects of n-Butanol Fraction of *G. latifolium* on some Endogenous Antioxidant Enzymes in the Kidney of CCl_4-Induced Kidney Damage Albino Rats

The effects of daily oral administration of n-butanol fraction of *G. latifolium* and silymarin for 28 days on the level of malondialdehyde (MDA) and some endogenous antioxidant enzymes (catalase, glutathione peroxidase and superoxide dismutase) of the kidney of CCl_4 induced kidney damage rats is represented in Table 5. The result showed that there was a significant (P<0.05) increase in the level of malondialdehyde (MDA) of the CCl_4-induced control rats compared with the normal control but, there was no significant (P>0.05) difference between the MDA levels of normal control and the induced treated groups. However the glutathione peroxidise (GPx), superoxide dismutase (SOD) and catalase (CAT) of CCl_4-induced control group were significantly (P<0.05) lowered than the normal control group but there was no significant (P>0.05) difference in the levels of endogenous antioxidant enzymes of all the induced treated groups when compared with the normal control group.

Table 5: Effects of Daily Doses of n-butanol fraction of *G. latifolium* on some Endogenous Antioxidant Enzymes in the Kidney of CCl_4-Induced Kidney Damage Albino Rats

Group (n=6)	MDA (µM)	SOD (U/ml)	CAT (U/ml)	GPx (mU/ml)
NC	1.30±0.06[a]	2.35±0.08[b]	47.3±1.41[b]	44.2±1.20[b]
VC	1.32±0.08[a]	2.28±0.08[b]	47.0±1.37[b]	44.7±0.99[b]
IC	2.35±0.14[b]	1.57±0.09[a]	34.2±1.30[a]	35.2±1.20[a]
CCl4 + BF	1.58±0.08[a]	2.15±0.08[b]	45.8±1.25[b]	44.8±0.91[b]
CCl4 + BF	1.55±0.08[a]	2.32±0.10[b]	46.7±1.36[b]	46.5±1.09[b]
CCl4 + BF	1.45±0.08[a]	2.33±0.09[b]	46.7±1.43[b]	46.3±1.15[b]
CCl4 + Std	1.47±0.09[a]	2.33±0.09[b]	44.8±0.95[b]	44.7±1.45[b]

Values are Means ± SEM. Values with different superscript down the columns are significantly different (P<0.05) NC: Normal Control rat, VC: Vehicle control rats, CCl_4: Carbon tetrachloride, IC: CCl_4 Induced kidney damage control rats, CCl_4 + BF: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+150mg/kg b.wt. of n-butanol fraction, CCl_4 + BF: CCl_4 Induced kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl_4 + Std: CCl_4 Induced kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin).MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

Histopathological Studies

Effects of n-Butanol Fraction of *G. latifolium* on the Kidneys

Plate 1 showed the histopathological examinations of the kidney sections of normal control, induction control and induced treated groups. The normal control group showed normal glomerulus and tubules. However, oxidative damage using CCl_4 resulted into intense glomerular and tubular necrosis. On daily oral administration of n-butanol fraction of *G. latifolium* methanolic leave extract and silymarin brought the kidneys back to moderate glomerular necrosis.

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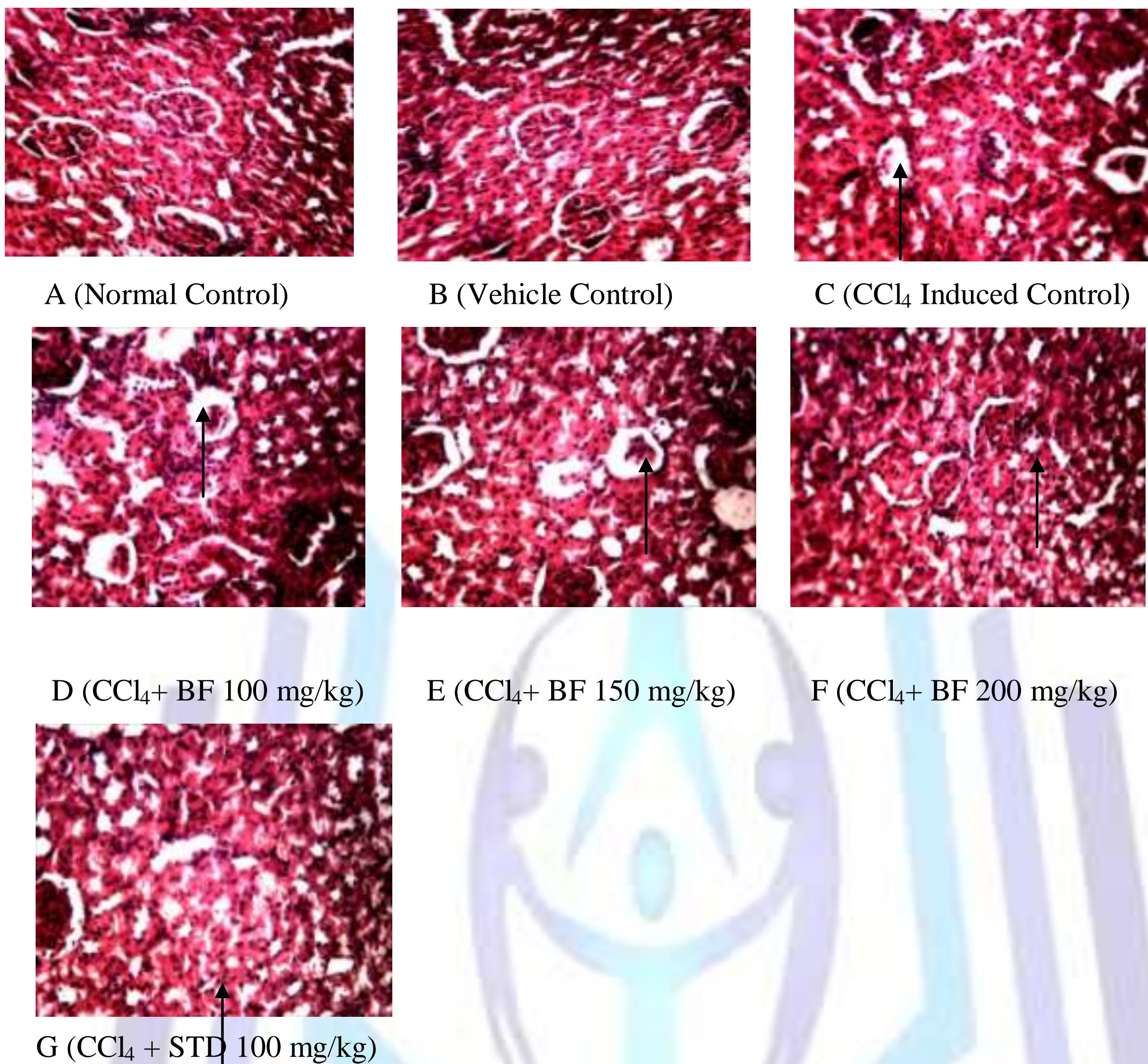

Plate 1: The Representative Kidney Region of CCl_4 Induced Kidney Damage Rats Treated with n-Butanol Fraction of *G. latifolium* and Silymarin (H&E STAIN X250)

A: Normal glomerulus and tubules

B: Normal glomerulus and tubules

C: Intense glomerular and tubular necrosis

D: Slight glomerular necrosis

E: Moderate glomerular necrosis

F: Moderate vacoulation and glomerular necrosis

G: Slight vacoulation.

DISCUSSION

The preliminary phytochemical studies revealed the presence of glycosides, saponins, tannins, alkaloids, and flavonoids in the crude methanolic leave extracts of *G. latifolium.* The presence of these phytochemicals in the plant, accounts for its usefulness as medicinal plant (Jayathilakan *et al.*, 2007). The quantitative phytochemical analysis showed that tannins had the highest concentration in the crude extract (Table 1) whereas the n-butanol fraction had the highest concentration of flavonoids, ascorbic acid and polyphenols (Venkatalakshmi *et al.,* 2012; Omonkhelin *et al.*, 2007) when compared to the ethylacetate fraction (Table 2). Plant phenolics, flavonoids and ascorbic acid constitute major groups of phytochemicals acting as primary *in vitro* antioxidants or free radical terminators (El-Sayed *et al.*, 2012). Therefore, it was reasonable to determine their concentration in the n-butanol and ethylacetate plant fractions with the aim of utilising the fraction with the highest concentration of *in vitro* antioxidant (Kumbhare *et al* 2012; Makepeace *et al.*, 1985). The potential health benefits associated with these phytochemicals has generated great interest among scientists for the development of natural *in vitro*

antioxidant compounds from plants (Rohman *et al.*, 2010; Masoumeh *et al.*, 2011; Wang *et al.*, 2008; Pazos *et al.*, 2005; Smith and Eyzaguine, 2007; Kumar *et al.*, 2009).

Haematological investigation provides information on the general pathophysiology of the blood and reticuloendothelial system (Baker and Silverton, 1985; Mishra *et al.*, 2009). Fairbarks (1967) showed that xenobiotics causes low PCV level which may be associated with the oxidization of sulphyldryl groups of the erythrocyte membrane thus, inflicting injury to the erythrocytes membrane. This is in agreement with the present study as packed cells volume (PCV) values in rats exposed to CCl_4 gave low levels of PCV. The n-butanol fraction appeared to boost blood cells as the values of PCV approached the normal control (Figure 1). This finding suggests that the administration of the n-butanol fraction of the methanolic leaves extract of *G. latifolium* to patient with remarkable low PCV level may increase their packed cell volume. It implies that the n-butanol fraction may possess constituents that would trigger the production of more blood cells (Patrick-Iwuanyanwu *et al.*, 2007; Emeka and Obioa, 2009).

Changes in the body weight after CCl_4 dosing have been used as a valuable index of CCl_4-related organ damage by (Bruckner *et al.*, 1986; Pradeep *et al.,* 2005) and thus, will be applicable in this study in order to justify the effects of CCl_4 on the body and organ weights of these animals. The decrease in changes in body weight (Figure 2) and consequent increase in kidney weights seen in CCl_4-induced control group was considered to be as a result of direct toxicity of CCl_4 and/or indirect toxicity that lead to kidney damage. This indicates that, CCl_4 may have induced hypertrophy of the cells of these organs as well as elicit remarkable tissue damage (Li *et al.*, 2011) which may have lead to the observed effects on the body and organ weights of these animals. However, all the induced treated groups experienced a significant increase in body weight changes as well as reduced change in organ weights, suggesting the possible curative effects of the n-butanol fraction of *G. latifolium* against kidney injury after CCl_4 induction.

The kidney helps in maintaining homeostasis of the body by reabsorbing important material and excreting waste products. Creatinine is a break down waste product formed in the muscle by creatine phosphate metabolism. Creatine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle for energy production. Creatinine retention in the blood is evidence of kidney impairment. Urea is the main end product of protein catabolism. Amino acid deamination takes place in the liver, which is also the site of urea cycle, where ammonia is converted into urea and excreted through urine. It represents 90% of the total urinary nitrogen excretion. Urea varies directly with protein intake and inversely with the rate of excretion. Renal diseases which diminish the glomerular filtration lead to urea retention. Administration of CCl_4 causes nephrotoxicity as indicated by significant elevation in serum level of creatinine (Figure 3) and urea (Figure 4). These results are in agreement with earlier findings by (Venkatanarayana *et al.*, 2012; Yacout *et al.*, 2012). From the present study it is evident that elevation in plasma urea and creatinine levels can be attributed to the damage of nephron structural integrity (Khan and Siddique, 2012). The different doses of n-butanol fraction significantly lowered urea and creatinine levels in the CCl_4-induced treated groups when compared with the CCl_4-induced not treated groups. This indicates that the n-butanol fraction of *G. latifolium* may improve renal function in kidney disease rats.

Antioxidant activity or scavenging activity of the generated free radicals is important in the curative effect of CCl_4-induced nephrotoxicity. The body has an effective defence mechanism to prevent and neutralize free radicals-induced damage. This is accomplished by a set of endogeneous antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. A non significant difference was observed in the kidney level of MDA in the normal control and all the induced treated groups however, there was an increase in the level of MDA in the CCl_4-induced control group when compared with the normal control and induced treated groups (Table 5). Also, there was a decrease in the activities of SOD, GPx and CAT in the CCl_4-induced control group when compared with the normal control and induced treated groups. This result suggests that the n-butanol fraction of *Gongronema latifolium* could improve renal function in animals as a result of its *in vitro* antioxidant potentials that may have assisted the endogenous enzymatic antioxidants to mop up free radicals generated by CCl_4. This can also be as a result of gradual restoration of the endogenous enzymatic antioxidant levels as less demand is placed on them thus, reversing the oxidative stress. This result is in agreement with the report of (Ragip *et al.,* 2008; Etim *et al.,* 2008; Ugochuwku *et al.*, 2003).

The histopathological findings of the kidney in the CCl_4- induced control group showed that CCl_4 caused an intense vascular congestion, vacoulation, lymphocyte hyperplasia and necrosis (Plate 1) thus, indicating its nephrotoxicity. This result is in agreement with (Venkatanarayana *et al.*, 2012). Following the administration of the n-butanol fraction of *Gongronema latifolium* and silymarin, the renal tubules and glomerulus showed moderate necrosis which may be as a result of regeneration and repair of the kidney cells (Emeka and Obioa, 2009; Etim *et al.*, 2008). Histopathological examinations are obviously in agreement with biochemical analysis.

CONCLUSIONS

The result of this study has scientifically justified the traditional use of *G. latifolium* in the management of several human diseases. The result showed that the n-butanol fraction of methanolic leave extract of *G. latifolium* possess *in vitro* antioxidants which may have contributed to its significant anti-nephrotoxic properties. The histological examination showed that the n-butanol fraction of *G. latifolium* has curative effect on the kidneys in CCl_4-induced kidney damage rats. The n-butanol fraction of *G. latifolium* is comparable to the standard drug (silymarin) in this study. This work provides the phytotherapeutic potential of n-butanol fraction of *G. latifolium* that may be useful to scientists and researchers in the nutraceutical industry.

RECOMMENDATIONS

1. There is need to carry out a bioactivity-guided fractionation, isolation and identification of the bioactive constituents of the n-butanol fraction which is responsible for the observed pharmacological activities.
2. There is need to carry out chronic toxicity studies of the n-butanol fraction of the plant so as to ascertain the safety of long term usage on the body.

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