

## Full Length Research Paper

## Effects of n-butanol fraction of *Gongronema latifolium* leave extract on some biochemical parameters in CCl<sub>4</sub>-induced oxidative damage in Wistar albino rats

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Effects of n-butanol fraction of *Gongronema latifolium* leave extract on some biochemical parameters in carbon tetrachloride-induced oxidative damage in Wistar albino rats were assessed. Fifty-four (54) Wistar albino rats were divided into treatment group and LD<sub>50</sub> groups. 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<sub>4</sub> (148 mg/kg) as a 1:1 (v/v) solution in olive oil and all the animals were fasted for 36 h. This was repeated once every week for a period of four (4) weeks. At the end of 28 days of treatment, liver marker enzymes studies showed that there was significant ( $p < 0.05$ ) increase in the serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin concentrations in CCl<sub>4</sub>-induced control group when compared with the normal control and induced treated groups but there was no significant ( $p > 0.05$ ) difference of these liver marker enzymes and bilirubin levels between the normal control and induced treated groups. Kidney function studies showed that there was significant ( $p < 0.05$ ) increase in creatinine and urea concentrations of CCl<sub>4</sub>-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 and liver antioxidant study revealed significant ( $p < 0.05$ ) decrease in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities as well as an increase in MDA concentration in CCl<sub>4</sub>-induced control rats when compared with the normal control rats. Histological section of the organs shows that the levels of hepatic and renal damage were higher in CCl<sub>4</sub>-induction control rats when compared with the normal control and induced treated groups. These findings suggest that n-butanol fraction of methanolic leaves extract of *G. latifolium* may have anti-hepatotoxic, anti-nephrotoxic and antioxidative effects against CCl<sub>4</sub>-induced liver and kidney damage in albino rats.

**Key words:** *Gongronema latifolium*, antioxidant, CCl<sub>4</sub>, liver, kidney.

### INTRODUCTION

*Gongronema latifolium* (Asclepiadaceae), is a perennial climber forest leafy vegetable with woody hollow glabrous 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

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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-hepatotoxic and anti-nephrotoxic properties (Okafor, 1989) and that flavonoids could protect the liver against oxidative injury induced by  $\text{CCl}_4$  *in vivo* (Akpan, 2004). Although many other plants have been reported to possess anti-hepatotoxic and anti-nephrotoxic 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 aim of this work was 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-hepatotoxic and anti-nephrotoxic activities of n-butanol fraction of methanolic leaves extract of *G. latifolium* against oxidative damage induced by  $\text{CCl}_4$  in Wistar albino rats.

## MATERIALS AND METHODS

### Chemicals/reagents

All assays kits were from Randox Laboratories Ltd. Ardmore, Co. Antrim 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 and normal saline were used.

### 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 h 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 (1 mm mesh size) and then concentrated in 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 2-4°C 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 min 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 in a water bath to afford four fractions (grams) respectively.

### Preliminary phytochemical screening

Test for glycosides, saponin, flavonoids, anthraquinones and tanins 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 and glycosides 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 total phenolic contents (TPC) using the Folin-Ciocalteu method adopted by Amin et al. (2004) was used. Ascorbic acid contents were determined using the method described by Barros et al. (2007). Determination of alkaloids was carried out using the procedure described by Harborne (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 after ethical approval was granted by the departmental ethical committee. 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 com-

mencement 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<sub>50</sub>) 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 10, 100 and 1000 mg of n-butanol fraction per kg body weight orally. They were observed for 24 h 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<sub>50</sub> 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, that is, 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 liver and kidney damage group of 6 rats each and LD<sub>50</sub> 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 148 mg/kg b.wt. carbon tetrachloride (CCl<sub>4</sub>) in olive oil. This serves as the CCl<sub>4</sub>-induced liver and kidney damage group (IC). Group D: Rats were treated with 148 mg/kg b.wt. CCl<sub>4</sub> in olive oil + 100 mg/kg b.wt. Silymarin as standard drug (CCl<sub>4</sub> + Std). Group E: Rats were treated with 148 mg/kg b.wt. CCl<sub>4</sub> in olive oil + 100 mg/kg b.wt. n-butanol fraction. (CCl<sub>4</sub> + BF). Group F: Rats were treated with 148 mg/kg b.wt. CCl<sub>4</sub> in olive oil + 150 mg/kg b.wt. n-butanol fraction (CCl<sub>4</sub>+BF). Group G: Rats were treated with 148 mg/kg b.wt. CCl<sub>4</sub> in olive oil + 200 mg/kg b.wt. n-butanol fraction (CCl<sub>4</sub>+BF).

### Induction of liver and kidney damage

The liver and kidney damage was induced by the administration of carbon tetrachloride (CCl<sub>4</sub>). Rats were injected intraperitoneally with a single dose of CCl<sub>4</sub> (148 mg/kg body weight) as a 1:1 (v/v) solution in olive oil and were fasted for 36 h 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 liver and kidney

Immediately after the blood was collected, the liver and kidneys were 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 liver and kidney were crushed in 50 mM 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. The homogenized organs were then centrifuged at 4000 rpm (2700 xg) for 15 min and the supernatant was collected using Pasteur pipette for further endogenous antioxidant activity assay. The percentage change in organ weight of each of the animals was calculated as follows:

$$\text{Change in weight (\%)} = \frac{\text{organ weight}}{\text{animal weight}} \times 100$$

### Haematological 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). Principle: The red blood cells are heavier than plasma with specific gravity of 1090 and 1030, respectively. When blood is placed in a capillary tube and centrifuge, they settle and are packed because of the centrifugal force acting on them. The volume occupied by the cells is measured with a haematocrit reader relative to the volume of the whole blood.

### Biochemical studies

Assessment of aspartate aminotransferase (AST) activity and assessment of alanine aminotransferase (ALT) activity were determined by the method described by Amador and Wacker (1962). Assessment of alkaline phosphatase (ALP) activity: Serum activity of ALP was determined by the method described by Haussament (1977). Determination of serum bilirubin concentration: The serum total and direct bilirubin was determined by the method of Jendrassik and Gróf (1938). Determination of total protein level: Total protein was determined colorimetrically according to the method described by Fine (1935). Determination of albumin level: The serum albumin was determined by the method of Doumas et al. (1971). 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 determine 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 is an adaptation of the method of Paglia and Valentine (1967). Estimation of thiobarbituric acid reactive substance (TBARS): 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 liver and kidneys of the animals were 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 liver and kidneys were made into permanent slides and examined under high (250x) resolution microscope with photographic facility and photomicrographs were taken.

**Table 1.** Quantitative analysis of the phytochemical constituents (mg/g) of *G. latifolium*.

Leave	Alkaloid (mg/g)	Saponin (mg/g)	Glycoside (mg/g)	Tannin (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*.

Fraction	Polyphenol (mg/g)	Flavonoid (mg/g)	Ascorbic acid (mg/g)
n- Butanol	4.53	5.15	2.24
Ethylacetate	2.39	4.51	0.62

### 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  $\pm$  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%).

### Qualitative phytochemical screening of the methanolic leaves extract of *G. Latifolium*

The qualitative phytochemicals screening of the methanolic leaves extract of *G. latifolium* revealed the presence of glycosides, alkaloids, saponin, flavonoids, tannins and the absence of free anthraquinone.

### Quantitative analysis of the phytochemical constituents (mg/g) of *G. latifolium*

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.

### Lethal dosage (LD<sub>50</sub>) 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 effects of sub-chronic oral administration of n-butanol fraction of *G. latifolium* methanolic leaves extract and silymarin (standard drug) at 100, 150 and 200 mg/kg b.wt. on packed cell volume in CCl<sub>4</sub>-induced liver and kidney damage in albino rats for 28 days is shown in Table 3. The result showed that the 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.

### Effects of n-butanol fraction of *G. latifolium* on body and organ weight change

Changes in body weight of rats induced liver and 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 4. The results showed no significant ( $P > 0.05$ ) difference in the body weight change of all the induced treated groups when compared with the normal control group. However, the CCl<sub>4</sub>-induced liver and kidney damage control group showed a significant ( $P < 0.05$ ) decrease in body weight when compared with the induced treated and normal control groups. Changes in organ weight of rats induced liver and 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 5. The result showed that there was no significant ( $P > 0.05$ ) difference between the percentage change in liver and

**Table 3.** Mean changes in PCV values of CCl<sub>4</sub>-induced liver and kidney damage rats treated daily with oral administration of n-butanol fraction of *G. latifolium* and silymarin (STD).

Groups (n=6)	PCV (%)	PCV (%)	PCV (%)	PCV (%)
	Week 1	Week 2	Week 3	Week 4
NC	47.3 ± 2.57 <sup>a</sup>	50.7 ± 0.72 <sup>b</sup>	50.2 ± 1.87 <sup>b</sup>	53.7 ± 0.42 <sup>bc</sup>
VC	45.8 ± 1.35 <sup>a</sup>	51.8 ± 0.48 <sup>bc</sup>	53.0 ± 1.16 <sup>bc</sup>	52.3 ± 0.92 <sup>b</sup>
IC	37.3 ± 1.78 <sup>b</sup>	28.7 ± 1.86 <sup>a</sup>	31.7 ± 2.26 <sup>a</sup>	31.0 ± 2.48 <sup>a</sup>
CCl <sub>4</sub> + BF	49.8 ± 1.92 <sup>a</sup>	52.5 ± 0.76 <sup>bc</sup>	54.7 ± 0.72 <sup>cd</sup>	56.2 ± 1.01 <sup>cd</sup>
CCl <sub>4</sub> + BF	48.7 ± 2.09 <sup>a</sup>	54.3 ± 0.80 <sup>c</sup>	57.7 ± 0.62 <sup>de</sup>	57.5 ± 0.76 <sup>de</sup>
CCl <sub>4</sub> + BF	49.3 ± 2.35 <sup>a</sup>	54.7 ± 1.31 <sup>c</sup>	61.0 ± 0.58 <sup>e</sup>	60.0 ± 0.58 <sup>e</sup>
CCl <sub>4</sub> + Std	49.3 ± 1.82 <sup>a</sup>	53.3 ± 0.49 <sup>bc</sup>	53.8 ± 1.54 <sup>bcd</sup>	56.3 ± 0.62 <sup>cd</sup>

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<sub>4</sub>: Carbon tetrachloride, IC: CCl<sub>4</sub> Induced Liver and Kidney damage control rats, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+150mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + Std: CCl<sub>4</sub> Induced liver and kidney damage rats+100 mg/kg b.wt. of standard drug (Silymarin).

**Table 4.** Mean changes in body weights of CCl<sub>4</sub>-induced liver and kidney damage rats treated daily with oral administration of n-butanol fraction of *G. latifolium* and silymarin (STD).

Groups (n=6)	Weight change	Weight change	Weight change	Weight change
	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)
NC	1.50 ± 0.22 <sup>b</sup>	2.83 ± 0.31 <sup>b</sup>	6.67 ± 0.67 <sup>b</sup>	10.3 ± 0.72 <sup>b</sup>
VC	1.50 ± 0.22 <sup>b</sup>	3.33 ± 0.21 <sup>b</sup>	6.50 ± 0.22 <sup>b</sup>	9.67 ± 0.56 <sup>b</sup>
IC	0.17 ± 0.54 <sup>a</sup>	0.83 ± 1.05 <sup>a</sup>	2.67 ± 1.05 <sup>a</sup>	4.00 ± 1.55 <sup>a</sup>
CCl <sub>4</sub> + BF	1.83 ± 0.17 <sup>b</sup>	3.50 ± 0.22 <sup>b</sup>	6.83 ± 0.48 <sup>b</sup>	10.5 ± 0.34 <sup>b</sup>
CCl <sub>4</sub> + BF	2.00 ± 0.26 <sup>b</sup>	3.67 ± 0.67 <sup>b</sup>	7.67 ± 0.84 <sup>b</sup>	11.5 ± 0.76 <sup>b</sup>
CCl <sub>4</sub> + BF	1.67 ± 0.21 <sup>b</sup>	3.00 ± 0.26 <sup>b</sup>	6.67 ± 0.62 <sup>b</sup>	10.2 ± 0.87 <sup>b</sup>
CCl <sub>4</sub> + Std	1.67 ± 0.21 <sup>b</sup>	3.50 ± 0.34 <sup>b</sup>	7.00 ± 0.37 <sup>b</sup>	10.5 ± 0.22 <sup>b</sup>

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<sub>4</sub>: Carbon tetrachloride, IC: CCl<sub>4</sub> Induced Liver and Kidney damage control rats, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+150mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + Std: CCl<sub>4</sub> Induced Liver and Kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin).

kidney weights of the entire induced treated group when compared with the normal control rats. However, the induced control rats present a significant (P<0.05) higher percentage change in kidney weights when compared with the normal control rats.

## Biochemical studies

### Assessment of liver function indices

Liver function indices of alanine aminotransferases (ALT), aspartate amino transferases (AST), alkaline phosphatases (ALP), total protein (TP), albumin (ALB) and bilirubin (DB and IB) concentrations in the serum of CCl<sub>4</sub>-induced liver and kidney damage rats after the daily oral administration of n-butanol fraction of *G. latifolium* and

silymarin for 28 days is represented in Tables 6 and 7. There was significant (P<0.05) increase in activities of all these liver marker enzymes (ALT, AST and ALP) in the CCl<sub>4</sub>-induced liver damage control group when compared with the normal control. The activities of ALT, AST and ALP in the induced treated groups were however significantly (P<0.05) reduced when compared with induced not treated group. The n-butanol fraction and silymarin significantly (P<0.05) increase the serum total protein levels of the induced treated groups when compared with the induced not treated group but there was no significant (P>0.05) difference between the serum total protein levels of all the induced treated groups and the normal control group. Also, serum albumin concentrations of the induced not treated group was significantly (P< 0.05) lower than the normal control and the induced treated

**Table 5.** Mean changes in organ weights of CCl<sub>4</sub>-induced kidney damage rats treated daily with oral administration of Silymarin and n-Butanol fraction of *G. latifolium*.

Groups (n=6)	Percentage change in kidney weight (g)	Percentage change in liver weight (g)
NC	0.52 ± 0.01 <sup>a</sup>	4.31 ± 0.14 <sup>a</sup>
VC	0.58 ± 0.02 <sup>a</sup>	4.58 ± 0.32 <sup>a</sup>
IC	0.85 ± 0.05 <sup>b</sup>	6.94 ± 0.38 <sup>b</sup>
CCl <sub>4</sub> + BF	0.57 ± 0.03 <sup>a</sup>	4.70 ± 0.17 <sup>a</sup>
CCl <sub>4</sub> + BF	0.52 ± 0.02 <sup>a</sup>	4.21 ± 0.53 <sup>a</sup>
CCl <sub>4</sub> + BF	0.51 ± 0.01 <sup>a</sup>	4.40 ± 0.14 <sup>a</sup>
CCl <sub>4</sub> + Std	0.55 ± 0.03 <sup>a</sup>	4.64 ± 0.14 <sup>a</sup>

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<sub>4</sub>: Carbon tetrachloride, IC: CCl<sub>4</sub> Induced Liver and Kidney damage control rats, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+150 mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + Std: CCl<sub>4</sub> Induced Liver and Kidney damage rats+100 mg/kg b.wt. of Standard Drug (Silymarin).

**Table 6.** Effects of daily doses of n-butanol fraction of *G. latifolium* on serum liver function parameters of CCl<sub>4</sub>-induced liver and kidney damage albino rats.

Group (n=6)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
NC	45.8 ± 2.85 <sup>a</sup>	42.5 ± 1.63 <sup>ab</sup>	60.2 ± 2.18 <sup>a</sup>
VC	44.5 ± 3.24 <sup>a</sup>	41.3 ± 1.89 <sup>a</sup>	59.5 ± 1.77 <sup>a</sup>
IC	60.3 ± 3.02 <sup>b</sup>	56.8 ± 2.18 <sup>c</sup>	76.0 ± 3.44 <sup>b</sup>
CCl <sub>4</sub> + BF	48.0 ± 2.15 <sup>a</sup>	47.3 ± 1.54 <sup>b</sup>	64.7 ± 2.33 <sup>a</sup>
CCl <sub>4</sub> + BF	47.3 ± 1.63 <sup>a</sup>	43.8 ± 1.74 <sup>ab</sup>	62.2 ± 1.11 <sup>a</sup>
CCl <sub>4</sub> + BF	45.5 ± 1.73 <sup>a</sup>	44.5 ± 1.71 <sup>ab</sup>	61.8 ± 1.78 <sup>a</sup>
CCl <sub>4</sub> + Std	48.3 ± 2.03 <sup>a</sup>	46.7 ± 1.71 <sup>ab</sup>	64.0 ± 1.81 <sup>a</sup>

Values are Means ± SEM. Values with different superscript down the column are significantly different (P<0.05). NC: Normal Control rat, VC: Vehicle control rats, CCl<sub>4</sub>: Carbon tetrachloride, IC: CCl<sub>4</sub> Induced Liver and Kidney damage control rats, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+150 mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+200 mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + Std: CCl<sub>4</sub> Induced Liver and Kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin). ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase.

**Table 7.** Effects of daily doses of n-butanol fraction of *G. latifolium* on serum total protein, albumin, direct and indirect bilirubin, creatinine and urea levels of CCl<sub>4</sub>-induced liver and kidney damage albino rats.

Group (n=6)	TP (g/dl)	ALB (g/dl)	DB (mg/dl)	IB (mg/dl)	CR (mg/dl)	Urea (mg/dl)
NC	61.2 ± 1.82 <sup>cd</sup>	37.2 ± 2.09 <sup>c</sup>	6.68 ± 0.41 <sup>a</sup>	5.30 ± 0.69 <sup>a</sup>	54.3 ± 1.65 <sup>a</sup>	3.28 ± 0.22 <sup>a</sup>
VC	60.3 ± 1.94 <sup>bcd</sup>	33.5 ± 1.52 <sup>bc</sup>	7.13 ± 0.68 <sup>a</sup>	5.10 ± 0.23 <sup>a</sup>	52.8 ± 3.26 <sup>a</sup>	3.55 ± 0.28 <sup>a</sup>
IC	44.8 ± 2.21 <sup>a</sup>	24.8 ± 1.74 <sup>a</sup>	10.5 ± 0.57 <sup>b</sup>	6.82 ± 0.24 <sup>b</sup>	63.8 ± 1.85 <sup>b</sup>	5.45 ± 0.16 <sup>b</sup>
CCl <sub>4</sub> + BF	55.2 ± 2.02 <sup>bc</sup>	32.2 ± 1.30 <sup>bc</sup>	7.70 ± 0.89 <sup>a</sup>	5.78 ± 0.25 <sup>ab</sup>	56.8 ± 1.35 <sup>a</sup>	3.78 ± 0.15 <sup>a</sup>
CCl <sub>4</sub> + BF	59.5 ± 1.48 <sup>bcd</sup>	29.5 ± 2.49 <sup>ab</sup>	7.62 ± 0.95 <sup>a</sup>	4.72 ± 0.24 <sup>a</sup>	55.5 ± 2.32 <sup>a</sup>	3.43 ± 0.14 <sup>a</sup>
CCl <sub>4</sub> + BF	63.3 ± 2.06 <sup>d</sup>	33.3 ± 1.71 <sup>bc</sup>	6.83 ± 0.78 <sup>a</sup>	5.55 ± 0.33 <sup>a</sup>	55.5 ± 2.32 <sup>a</sup>	3.38 ± 0.11 <sup>a</sup>
CCl <sub>4</sub> + Std	54.5 ± 2.45 <sup>b</sup>	33.0 ± 1.93 <sup>bc</sup>	8.23 ± 0.62 <sup>a</sup>	4.83 ± 0.51 <sup>a</sup>	58.0 ± 1.29 <sup>ab</sup>	3.82 ± 0.15 <sup>a</sup>

Values are Means ± SEM. Values with different superscript down the column are significantly different (P<0.05). NC: Normal control rat, VC: Vehicle control rats, CCl<sub>4</sub>: Carbon tetrachloride, IC: CCl<sub>4</sub> Induced Liver and Kidney damage control rats, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+150mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + Std: CCl<sub>4</sub> Induced Liver and Kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin). TP: Total protein, ALB: Albumin, DB: Direct bilirubin, IB: Indirect bilirubin, CR: Creatinine.

**Table 8.** Effects of daily doses of n-butanol fraction of *G. latifolium* on some endogenous antioxidant enzymes in the liver of CCl<sub>4</sub>-induced liver and kidney damage albino rats.

Group (n=6)	MDA (μM)	SOD (U/ml)	CAT (U/ml)	GPx (mU/ml)
NC	1.32 ± 0.06 <sup>a</sup>	2.45 ± 0.08 <sup>b</sup>	50.0 ± 2.93 <sup>b</sup>	50.8 ± 1.17 <sup>b</sup>
VC	1.52 ± 0.07 <sup>a</sup>	2.25 ± 0.08 <sup>b</sup>	51.2 ± 2.06 <sup>b</sup>	49.5 ± 1.89 <sup>b</sup>
IC	2.53 ± 0.09 <sup>b</sup>	1.43 ± 0.09 <sup>a</sup>	40.3 ± 1.17 <sup>a</sup>	37.7 ± 1.67 <sup>a</sup>
CCl <sub>4</sub> + BF	1.53 ± 0.11 <sup>a</sup>	2.23 ± 0.10 <sup>b</sup>	52.0 ± 1.27 <sup>b</sup>	47.2 ± 1.64 <sup>b</sup>
CCl <sub>4</sub> + BF	1.52 ± 0.11 <sup>a</sup>	2.45 ± 0.09 <sup>b</sup>	53.0 ± 1.32 <sup>b</sup>	49.8 ± 1.30 <sup>b</sup>
CCl <sub>4</sub> + BF	1.48 ± 0.13 <sup>a</sup>	2.43 ± 0.09 <sup>b</sup>	54.5 ± 1.18 <sup>b</sup>	51.0 ± 1.53 <sup>b</sup>
CCl <sub>4</sub> + Std	1.58 ± 0.10 <sup>a</sup>	2.35 ± 0.11 <sup>b</sup>	52.5 ± 1.34 <sup>b</sup>	49.83 ± 2.34 <sup>b</sup>

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<sub>4</sub>: Carbon tetrachloride, IC: CCl<sub>4</sub> Induced liver and kidney damage control rats, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced liver and kidney damage rats+100 mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+150 mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + Std: CCl<sub>4</sub> Induced Liver and Kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin). MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase.

groups, but there was no significant (P>0.05) difference between the serum albumin levels of all the induced treated groups and the normal control group. Also, the levels of bilirubin in the induced treated groups were however significantly (P<0.05) reduced when compared with induced not treated group, but there was no significant (P>0.05) difference between the bilirubin levels of all the induced treated groups and the normal control group.

#### Effects of n-butanol fraction of *G. latifolium* on kidney function parameters

Creatinine concentrations in the serum of normal and CCl<sub>4</sub> induced liver and kidney damage rats after the oral administration of n-butanol fraction of *G. latifolium* and silymarin for 28 days is presented in Table 7. The results showed that the concentration of creatinine in the serum of CCl<sub>4</sub> 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. Also, the urea concentrations in the serum of normal and CCl<sub>4</sub>-induced liver and kidney damage rats after the oral administration of n-butanol fraction of *G. latifolium* and silymarin for 28 days (Table 7) showed that the concentration of urea in the serum of CCl<sub>4</sub>-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.

#### *In vivo* antioxidant studies

##### **Effects of n-butanol fraction of *G. latifolium* on some endogenous antioxidant enzymes in the liver of CCl<sub>4</sub>-induced liver and 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 liver of CCl<sub>4</sub>-induced liver and kidney damage rats is represented in Table 8. There was a significant (P<0.05) increase in the level of MDA and a significant (P<0.05) decrease in the level of CAT, glutathione peroxidase (GPx) and superoxide dismutase (SOD) of the CCl<sub>4</sub>-induced liver and kidney damage control rats when compared with the normal control. There was no significant (P>0.05) difference in the levels of MDA and endogenous antioxidant enzymes of all the induced treated groups when compared with the normal control group.

##### **Effects of n-butanol fraction of *G. latifolium* on some endogenous antioxidant enzymes in the kidney of CCl<sub>4</sub>-induced liver and 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 MDA and some endogenous antioxidant enzymes (catalase, glutathione peroxidase and superoxide dismutase) of the liver of CCl<sub>4</sub> induced liver and kidney damage rats is presented in Table 9. The result showed that there was a significant (P<0.05) increase in the level of MDA of

**Table 9.** Effects of daily doses of n-butanol fraction of *G. latifolium* on some endogenous antioxidant enzymes in the kidney of CCl<sub>4</sub>-induced liver and kidney damage albino rats.

Group (n=6)	MDA (µM)	SOD (U/ml)	CAT (U/ml)	GPx (mU/ml)
NC	1.30 ± 0.06 <sup>a</sup>	2.35 ± 0.08 <sup>b</sup>	47.3 ± 1.41 <sup>b</sup>	44.2 ± 1.20 <sup>b</sup>
VC	1.32 ± 0.08 <sup>a</sup>	2.28 ± 0.08 <sup>b</sup>	47.0 ± 1.37 <sup>b</sup>	44.7 ± 0.99 <sup>b</sup>
IC	2.35 ± 0.14 <sup>b</sup>	1.57 ± 0.09 <sup>a</sup>	34.2 ± 1.30 <sup>a</sup>	35.2 ± 1.20 <sup>a</sup>
CCl <sub>4</sub> + BF	1.58 ± 0.08 <sup>a</sup>	2.15 ± 0.08 <sup>b</sup>	45.8 ± 1.25 <sup>b</sup>	44.8 ± 0.91 <sup>b</sup>
CCl <sub>4</sub> + BF	1.55 ± 0.08 <sup>a</sup>	2.32 ± 0.10 <sup>b</sup>	46.7 ± 1.36 <sup>b</sup>	46.5 ± 1.09 <sup>b</sup>
CCl <sub>4</sub> + BF	1.45 ± 0.08 <sup>a</sup>	2.33 ± 0.09 <sup>b</sup>	46.7 ± 1.43 <sup>b</sup>	46.3 ± 1.15 <sup>b</sup>
CCl <sub>4</sub> + Std	1.47 ± 0.09 <sup>a</sup>	2.33 ± 0.09 <sup>b</sup>	44.8 ± 0.95 <sup>b</sup>	44.7 ± 1.45 <sup>b</sup>

Values are Means ± SEM. Values with different superscript down the column are significantly different (P<0.05). NC: Normal Control rat, VC: Vehicle control rats, CCl<sub>4</sub>: Carbon tetrachloride, IC: CCl<sub>4</sub> Induced Liver and Kidney damage control rats, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and kidney damage rats+100mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+150mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + BF: CCl<sub>4</sub> Induced Liver and Kidney damage rats+200mg/kg b.wt. of n-butanol fraction, CCl<sub>4</sub> + Std: CCl<sub>4</sub> Induced Liver and Kidney damage rats+100mg/kg b.wt. of Standard Drug (Silymarin). MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

the CCl<sub>4</sub>-induced control rats when 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 GPx, SOD and CAT of CCl<sub>4</sub> 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.

## Histopathological studies

### Effects of n-butanol fraction of *G. latifolium* on liver and kidney

The histological section of the liver and kidneys of CCl<sub>4</sub>-induced oxidative damage rats treated with n-butanol fraction of *G. latifolium* methanolic leave extract and silymarin for 28 days is shown in Plates 1 and 2. The histopathological examinations of liver section of normal control group showed normal cellular architecture with distinct hepatic cells. CCl<sub>4</sub>-induced control group liver showed an intense hepatic necrosis with vascular congestion, vacuolation, lymphocyte hyperplasia and degeneration of normal hepatic cells. The induced treated groups showed almost normalization of the hepatic cells after administration of n-butanol fraction of *G. latifolium*.

Also, the histopathological examinations of kidney section of normal control group showed normal glomerulus and tubules. However, oxidative damage using CCl<sub>4</sub> resulted into intense glomerular and tubular necrosis. Daily oral administration of n-butanol fraction of *G. latifolium* methanolic leave extract and silymarin brought the kidneys back to moderate glomerular necrosis.

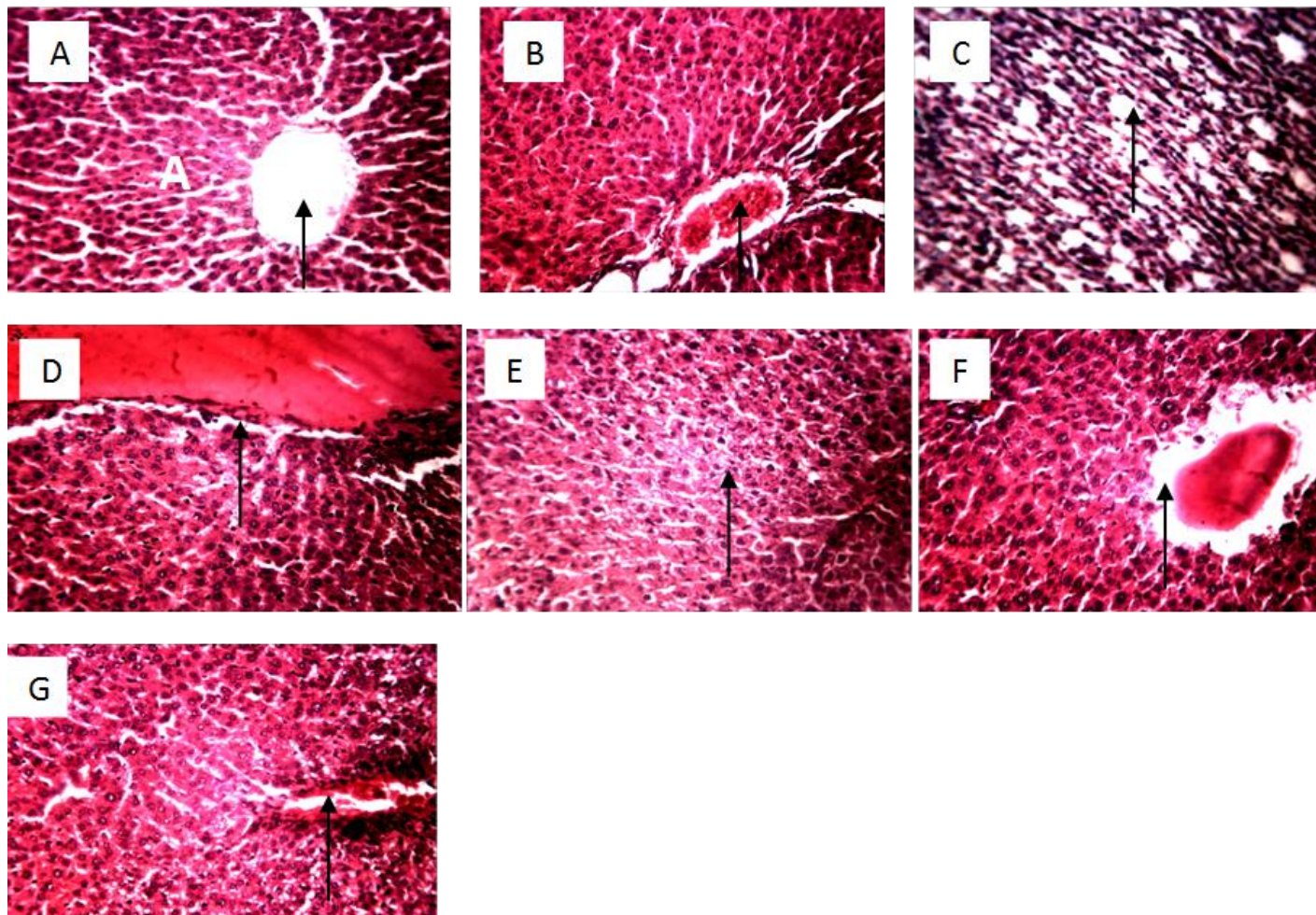
## DISCUSSION

The preliminary phytochemical studies revealed the pre-

sence 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 with 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). Polyphenols, flavonoids and ascorbic acid scavenging potentials and metal chelating ability is (Wang et al., 2008) dependent upon their unique structure, the number and position of the hydroxyl groups (Pazos et al., 2005; Smith and Eyzaguine, 2007; Kumar et al., 2009). 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).

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 sulphhydryl 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<sub>4</sub> gave low levels of PCV. The n-butanol fraction appeared to





**Plate 1.** The Representative Liver Region of  $\text{CCl}_4$ -Induced Liver and Kidney damage rats treated with n-butanol fraction of *G. latifolium* and silymarin (H&E STAIN X250). Group A: Normal Control, Group B: Vehicle Control, Group C:  $\text{CCl}_4$ -Induced Control, Group D:  $\text{CCl}_4$  + STD 100 mg/kg, Group E:  $\text{CCl}_4$ + BF 100 mg/kg, Group F:  $\text{CCl}_4$ + BF 150 mg/kg, Group G:  $\text{CCl}_4$ + BF 200 mg/kg.

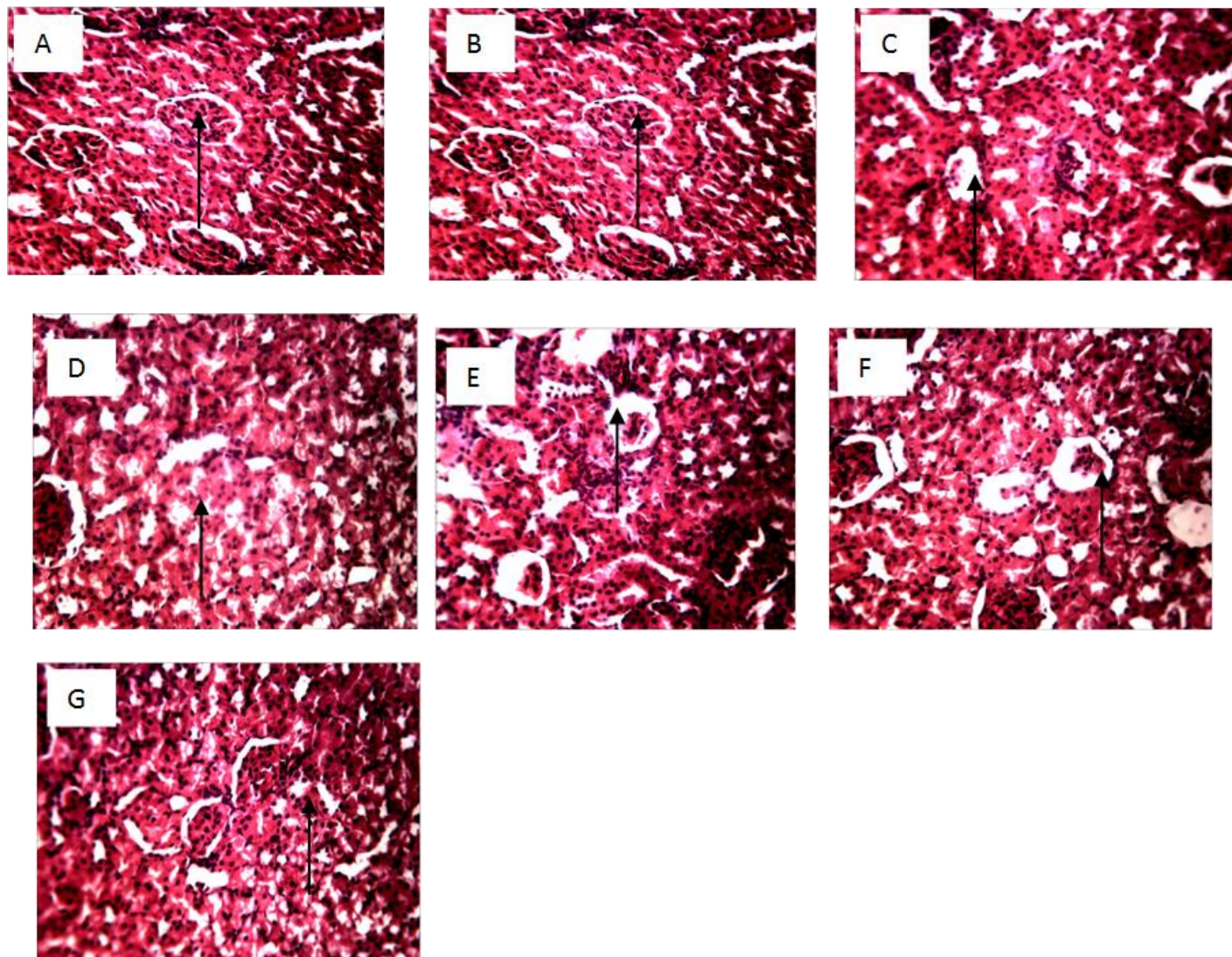
boost blood cells as the values of PCV approached the normal control (Table 3). This finding suggests that the administration of the n-butanol fraction of the methanolic leaves extract of *G. latifolium* to patient with remarkable low PCV level may increase their packed cell volume. It implies that the 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  $\text{CCl}_4$  dosing have been used as a valuable index of  $\text{CCl}_4$ -related organ damage by Bruckner et al. (1986) and Pradeep et al. (2005) and thus, will be applicable in this study in order to justify the effects of  $\text{CCl}_4$  on the body and organ weights of these animals. The decrease in changes in body weight (Table 4) and consequent increase in liver and kidney weights (Table 5) seen in  $\text{CCl}_4$ -induced control group was considered to be as a result of direct toxicity of  $\text{CCl}_4$  and/or indirect toxicity that lead to liver and kidney damage. This indicates that,  $\text{CCl}_4$  may have induced hyper-

trophy 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 liver and kidney injury after  $\text{CCl}_4$  induction.

Assessment of liver can be made by estimating the activities of serum ALT, AST and ALP which are enzymes originally present at higher concentration in cytoplasm (Reham et al., 2009). When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage (Nkosi et al., 2005; Dominic et al., 2012). Administration of  $\text{CCl}_4$  caused a significant ( $P < 0.05$ ) elevation of these liver marker enzyme levels and a consequent decrease in the level of serum proteins when compared with normal control group (Table 6). The elevated level of these liver marker enzymes with a corres-





**Plate 2.** The Representative Kidney Region of  $\text{CCl}_4$ -Induced Liver and Kidney damage rats treated with n-butanol fraction of *G. latifolium* and silymarin (H&E STAIN X250). Group A: Normal Control, Group B: Vehicle Control, Group C:  $\text{CCl}_4$ -Induced Control, Group D:  $\text{CCl}_4$  + STD 100 mg/kg, Group E:  $\text{CCl}_4$ + BF 100 mg/kg, Group F:  $\text{CCl}_4$ + BF 150 mg/kg, Group G:  $\text{CCl}_4$ + BF 200 mg/kg.

ponding decrease in serum protein levels observed in the  $\text{CCl}_4$ -induced not treated group corresponded to extensive liver damage induced by  $\text{CCl}_4$  which may be as a result of an impaired protein turnover (Table 7). These results are in agreement with previous finding that the activity levels of serum ALT, ALP and AST were significantly elevated and there was a significant decrease in serum protein levels in rats after  $\text{CCl}_4$  administration (Khan et al., 2012; Battu et al., 2012; Shahid et al., 2012; Etim et al., 2008).

Also, the significant ( $P < 0.05$ ) elevation of bilirubin levels in the  $\text{CCl}_4$ -induced not treated group when compared with the normal control and the induced treated groups (Table 7) may be as a result of haemolytic anaemia that may be associated with oxidative damage to red blood cells thus, leading to elevated bilirubin level

since bilirubin is an intermediate product in haemoglobin breakdown in the liver (Reham et al., 2009). Again, this elevated bilirubin level may also be associated with reduced hepatocyte uptake of bilirubin, impaired conjugation of bilirubin and reduced hepatocyte secretion of bilirubin (Table 7) (Nkosi et al., 2005; Dominic et al., 2012). Also, there are significant elevation of direct (conjugated) and indirect (unconjugated) bilirubin levels in the blood serum of  $\text{CCl}_4$ -induced not treated group which may be attributed to the inability of the hepatocyte to secrete conjugated bilirubin as envisioned in elevated direct bilirubin level or may also be due to obstruction in the flow of bile from the bile duct as a result of severe liver damage (Emeka and Obioa, 2009). Furthermore, elevated indirect bilirubin level may be as a result of liver necrosis which is capable of causing the liver not to

conjugate bilirubin and may also cause the hepatocytes to lose its ability to take up bilirubin (Khan et al., 2012; Battu et al., 2012; Shahid et al., 2012).

There was significant ( $P < 0.05$ ) restoration of these liver marker enzymes levels as well as bilirubin and serum proteins levels on administration of the n-butanol fraction and silymarin for 28 days at a dose of 100, 150 and 200 mg/kg b.wt. The reversal of these serum liver marker enzymes in  $\text{CCl}_4$ -induced treated groups towards a near normalcy by the n-butanol fraction observed in this study may be due to the prevention of the leakage of these intracellular enzymes as a result of the presence of polyphenols, flavonoids and ascorbic acid in the n-butanol fraction as well as their membrane stabilizing activity which may be attributed to their ability to mop up free radicals that attack cell membranes. Also, the repeated contact of these *in vitro* antioxidants with hepatocytes may lead to increased stability of the cell membrane (Muthu et al., 2008; Chavan et al., 2012). Again, the ability of the n-butanol fraction to reduce the bilirubin level to near normalcy may be as a result of its ability to assist in reducing oxidative damage to red blood cells which may lead to reduction in haemoglobin breakdown by the liver. This is in agreement with the commonly accepted view that serum levels of transaminases, bilirubin and serum proteins returns to normalcy with the healing of hepatic parenchyma cells as well as the regeneration of hepatocytes (Chavan et al., 2012; Ugwu and Eze, 2010). It is therefore, a clear manifestation of the hepatocurative effects of the n-butanol fraction of *G. latifolium*.

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  $\text{CCl}_4$  causes nephrotoxicity as indicated by significant ( $P < 0.05$ ) elevation in serum level of creatinine and urea (Table 7). These results are in agreement with earlier findings (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 ( $P < 0.05$ ) lowered urea and creatinine levels in the  $\text{CCl}_4$ -induced treated groups when compared with the  $\text{CCl}_4$ -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  $\text{CCl}_4$ -induced hepatotoxicity. The body has an effective defence mechanism to prevent and neutralize free radicals-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury (Mohajeri et al., 2011; Tamilarasi et al., 2012). The increased level of MDA in the liver tissue of the rats administered  $\text{CCl}_4$  (Table 8) may be as a result of the enhanced membrane lipid peroxidation by free radicals generated and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals (Liu et al., 2009; Kim et al., 2010; Khan et al., 2012). Also, the decreased activity of SOD, GPx and CAT in the liver tissues of  $\text{CCl}_4$ -induced rats may be due to high concentration of these free radicals generated by  $\text{CCl}_4$  which may lead to decreased level or inactivation of these endogenous antioxidant enzymes (Showkat et al., 2010). Treatment with n-butanol fraction of *G. latifolium* significantly ( $P < 0.05$ ) increased the levels of SOD, GPx and CAT activities and a consequent significant ( $P < 0.05$ ) reduction in MDA. The effects of the n-butanol fraction were comparable to the standard drug (Silymarin). Thus, this result suggests that n-butanol fraction of *G. latifolium* contains free radical scavenging activity due to the presence of *in vitro* antioxidants, which could exert beneficial action against pathophysiological alterations caused by the presence of superoxide and hydroxide free radicals as well as hydrogen peroxide indicating the regeneration of damaged liver cells (Etim et al., 2008; Ugochuwku et al., 2003).

Furthermore, a non significant ( $P > 0.05$ ) difference was observed in the kidney level of MDA (Table 9) in the normal control and all the induced treated groups in contrast to a significant increase in the level of MDA in the  $\text{CCl}_4$ -induced control group. Also, there was significant decrease in the activities of SOD, GPx and CAT in the  $\text{CCl}_4$ -induced control group as compared to the normal control and induced treated groups that showed elevated activities of these endogenous antioxidant enzymes. This suggests that, the n-butanol fraction of *G. latifolium* could improve renal function in animals due to its *in vitro* antioxidant potentials that may have assisted the endogenous enzymatic antioxidants to mop up free radicals generated by  $\text{CCl}_4$  or could 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) and Ugochuwku et al. (2003).

The histopathological studies of the liver and kidneys in the  $\text{CCl}_4$ -induced control group showed that  $\text{CCl}_4$  caused an intense vascular congestion, vacuolation, lymphocyte

hyperplasia and necrosis (Plates 1 and 2) indicating its hepatotoxicity and nephrotoxicity. This result is in agreement with that of Venkatanarayana et al. (2012). Following the administration of the n-butanol fraction of *G. latifolium* and silymarin, the hepatocytes showed close to normal cellular architecture while the glomerulus showed moderate necrosis which may be as a result of regeneration and repair of liver and kidney cells (Emeka and Obioa, 2009; Etim et al., 2008).

In line with these findings, it is obvious that histopathological examinations are in agreement with observed biochemical analysis. This result is in agreement with the report of Etim et al. (2008) and Ugochuwku et al. (2003). It was demonstrated that liver is not the only target organ of CCl<sub>4</sub>, it also causes free radical generation in other organs, such as heart, lung, testis, brain and blood (Khan and Ahmed, 2009; Khan et al., 2009; Khan and Zehra, 2011).

## Conclusions

The result of this study has scientifically justified the traditional use of *G. latifolium* in the management of 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-hepatotoxic properties. The histological examination showed that the n-butanol fraction of *G. latifolium* has curative effect on the liver in CCl<sub>4</sub> induced liver damage rats. The n-butanol fraction of *G. latifolium* is comparable to the standard drug (silymarin). 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 animals.

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