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Original article:

HEPATOPROTECTIVE EFFECT OF AN AQUEOUS EXTRACT OF THE LEAVES OF *ACALYPHA WILKESIANA* 'GODSEFFIANA' MUELL ARG (EUPHORBIACEAE) AGAINST CARBON TETRACHLORIDE INDUCED LIVER INJURY IN RATS

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ABSTRACT

The potential of aqueous extract of the leaves of *Acalypha wilkesiana*, to protect against carbon tetrachloride induced liver damage was investigated in Wistar albino rats. The carbon tetrachloride was prepared 1:5 (v:v) in olive oil, and administered subcutaneously at 1 mL/kg body weight. The extract was administered to both normal and carbon tetrachloride treated rats at 100, 200 and 300 mg/kg. On fractionation and gas chromatographic analysis of the crude aqueous extract, thirty nine known alkaloids were detected, consisting mainly of akuamidine (69.027 %), voacangine (26.226 %), echitamine (1.974 %), echitamidine (0.599 %), lupanine (0.521 %) and augustamine (0.278 %). Compared to test control, the treatment dose dependently produced significantly lower (P<0.05) alkaline phosphatase, aspartate and alanine transaminase activities. Histopathological studies on the liver sections showed that pre-treatment with the extract protected against carbon tetrachloride induced fatty degeneration of hepatocytes, thus, confirming the results of the biochemical studies. The above results imply that treatment with the plant extract protects the liver against carbon tetrachloride induced hepatotoxicity, therefore, justifying the use of *Acalypha wilkesiana* in African traditional health care for the management of liver problems.

Keywords: *Acalypha wilkesiana* 'Godseffiana' Muell Arg (Euphorbiaceae), carbon tetrachloride, histopathology, hepatospecific markers

INTRODUCTION

Acalypha wilkesiana, a member of the spurge family (Euphorbiaceae), belongs to the genus Acalypha comprising about 570 species (Riley, 1963, as cited in Ogundaini, 2005). A large proportion of members of this genus are weeds while the others are ornamental plants. *Acalypha wilkesiana* is commonly called copperleaf, Joseph's coat, fire dragon, beef steak plant and match-me-if-you-can (Christman, 2004). Although native to Fiji and nearby islands in the South Pacific, it has spread to most parts of the world, especially the tropics of Africa, America and Asia. It is a popular outdoor plant that provides colour throughout the year, although it is also grown indoors as a container plant. Many cultivars are available with different leaf forms and colours: *A. wilkesiana* 'Godseffiana' has narrow, drooping, green leaves with creamy-white margins; 'Marginata' has coppery-green leaves with pink or crimson margins; 'Macrophylla' has larger leaves, variegated with bronze, cream, yellow and red; while 'Musaica' has green leaves that are mottled with orange and red (Gilman, 1999; Christman, 2004). In Southern Nigeria, the expressed juice or boiled decoction of the leaves of *A. wilkesiana* cv. Godseffiana is

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used in traditional health care practice, for the management of gastrointestinal disorders, fungal skin infections, hypertension and diabetes mellitus. The leaf-poultice is used in the treatment of headache, swellings, colds and malaria (Akinyemi et al., 2005). The seeds of *A. wilkesiana* are used in compounding a complex plant mixture used by traditional healers in South-West Nigeria to treat breast tumours and inflammation (Bussing et al., 1999; Taraphdar et al., 2001). The antimicrobial (Akinyemi et al., 2005; Ogundaini, 2005; Oladunmoye, 2006), hypolipidaemic (Ikewuchi, 2010; Ikewuchi and Ikewuchi, 2010), diuretic (Ikewuchi et al., 2009a), hypoglycaemic (Ikewuchi et al., 2009a, 2011a) and antihypertensive (Ikewuchi et al., 2009b, 2011b) properties of the leaves have been reported.

Earlier analysis of the leaves of *Acalypha wilkesiana* revealed the presence of sesquiterpenes, monoterpenes, triterpenoids, polyphenols, gallic acid, corilagin, geraniin, quercetin 3-O-rutinoside, kaempferol 3-O-rutinoside, saponins, tannins, anthroquinone and glycosides (Akinde, 1986; Adesina et al., 2000; Oladunmoye, 2006). They were also found to be were rich in some potent hepatoprotective agents like vitamin C (Ikewuchi and Ikewuchi, 2009), flavonoids and tannic acid (Ikewuchi et al., 2010, 2011a). Therefore, in this study, the ability of an aqueous extract of the leaves of *A. wilkesiana* 'Godseffiana' Muell Arg, to protect against carbon tetrachloride induced liver damage was investigated in Wistar albino rats.

MATERIALS AND METHODS

Collection of plant samples and preparation of plant extract

Samples of the fresh *Acalypha wilkesiana* plants (Figure 1) were collected from within the Choba and Abuja Campuses of the University of Port Harcourt, Nigeria. After due identification at the University of Port Harcourt Herbarium, Port Harcourt, they were cleaned, before removing their leaves, which were then oven dried at 55 °C and ground into powder. The resultant powder was soaked in hot distilled water for 12 h, after which the resultant mixture was filtered and the filtrate (hereinafter referred to as the aqueous extract) was stored in the refrigerator for subsequent use. A known volume of this extract was evaporated to dryness, and the weight of the residue obtained, was used to determine the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract. The resultant crude aqueous residue was subjected to phytochemical analysis.

Figure 1: *Acalypha wilkesiana* Muell Arg

Determination of the phytochemical content of the crude aqueous leaf extract

Calibration, identification and quantification

Standard solutions were prepared in methanol. The linearity of the dependence of response on concentration was verified by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound determined, using the standards.

Determination of alkaloid composition

The extraction of the alkaloids was carried out according to the method of Tram et al. (2002). The residue from the aqueous extract above, was extracted with methanol and subjected to gas chromatographic analysis. Three grams of the crude aqueous extract was extracted with 25 mL of methanol for 6 h at room temperature, before fil-

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tration. The filtrate was concentrated in a rotary evaporator, before "drying off" water using anhydrous sodium sulphate, prior to gas chromatography analysis. Gas chromatographic analyses was carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01 [1206] software, to quantify and identify compounds. The column was a capillary DB-5MS (30 m × 0.25 mm × 0.25 µm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 28 psi and 38 psi. The oven was programmed as follows: initial temperature at 60 °C for 5 min; first ramping at 10 °C/min for 20 min, followed by a second ramping at 15 °C/min for 4 min. The chromatogram of the extract is shown in Figure 2.

Experimental design for the hepatoprotective study

Wistar albino rats (180-200 g) were collected from the animal house of the Department of Physiology, University of Nigeria, Enugu Campus. Studies were conducted in compliance with applicable laws and regulations for handling experimental animals. The rats were weighed and sorted into eight groups (Table 1) of five animals each, so that their average weights were approximately equal. The animals were housed in plastic cages at the animal house of the Department of Biochemistry, University of Port Harcourt. After a one-week acclimatization period on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), the treatment commenced. The extract was administered orally on daily basis for eight days. The dosage of administration of the extract was adapted, with modification, from Ikewuchi and Ikewuchi (2010) and Ikewuchi (2010). The carbon tetrachloride was prepared 1:5 (v:v) in olive oil, and administered subcutaneously at 1 mL/kg body weight of carbon tetrachloride, on days 4 and 8. The dosage and method of administration of carbon tetrachloride was adapted from Obi and Uneh (2003), with modification. Twenty four hours after the last administration of carbon tetrachloride, the rats were weighed and anaesthetized by exposure to chloroform. While under anesthesia, they were painlessly sacrificed and blood was collected from each rat into heparin sample bottles, after which the livers were collected and preserved in 10 % formalin, for histochemical analysis. The heparin anti-coagulated blood samples were centrifuged at 1000 g for 10 min, after which their plasma were collected and stored for subsequent analysis.

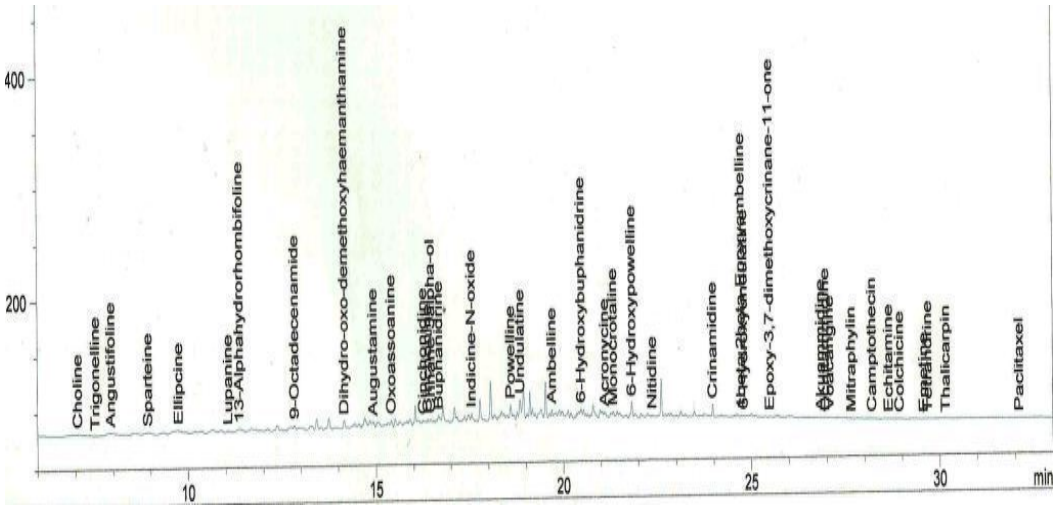

Figure 2: Gas chromatogram of the alkaloid composition of an aqueous extract of *Acalypha wilkesiana* leaves

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Table 1: Experimental design for the hepatoprotective screening

S/N	ID	Treatment
1	Normal	Olive oil (1 mL/kg) and normal saline and Water
2	Test control	Carbon tetrachloride (1 mL/kg) and water
3	Treatment control I (AWC1)	Olive oil (1 mL/kg) and extract (100 mg/kg)
4	Treatment control II (AWC2)	Olive oil (1 mL/kg) and extract (200 mg/kg)
5	Treatment control III (AWC3)	Olive oil (1 mL/kg) and extract (300 mg/kg)
6	Treatment I (AW1)	Carbon tetrachloride (1 mL/kg) and extract (100 mg/kg)
7	Treatment II (AW2)	Carbon tetrachloride (1 mL/kg) and extract (200 mg/kg)
8	Treatment III (AW3)	Carbon tetrachloride (1 mL/kg) and extract (300 mg/kg)

Determination of plasma hepatospecific markers

The plasma activities of alanine and aspartate transaminases, and alkaline phosphatase were determined using Randox test kits (Randox Laboratories Ltd., Crumlin, England, UK). The activities of alanine and aspartate transaminases were respectively measured by monitoring at 546 nm the concentrations of pyruvate and oxaloacetate hydrazones formed with 2,4-dinitrophenylhydrazine. The activity of alkaline phosphatase was determined by monitoring the degradation of p-nitrophenylphosphate to p-nitrophenol, at 405 nm.

Plasma total bilirubin and protein concentrations were determined using Randox test kits (Randox Laboratories Ltd., Crumlin, England, UK). The wavelength for the determination of total bilirubin was 578 nm, while that of total protein was 560 nm.

Determination of percentage protection (% protection)

The percentage protection provided by the extract against carbon tetrachloride induced liver damage was calculated using the following formula adapted from Al-Qarawi *et al.* (2004).

$$\%\,\mathbf{Protection} = \frac{(Parameter_{Test\ control} - Parameter_{Treatment})}{Parameter_{Test\ control} - Parameter_{Control}} \times 100 \qquad [Eqn\ 1]$$

Histopathological study on the liver

The histopathology study on the liver samples was carried out by Professor S.O. Nwosu, from the Department of Anatomical Pathology, University of Port Harcourt Teaching Hospital. Small pieces of liver tissues were collected in 10 % formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6 µm in thickness were cut, mounted on slide and stained with hematoxylin and eosin. The sections were then examined via light microscopy (Opticphot-2; Nikon, Tokyo, Japan) at x100 magnification.

Statistical analysis of data

All values are quoted as the mean ± s.e.m. (standard error in the mean). The values of the various parameters were analyzed for statistical significant differences between the groups, using the Student's *t*-test, with the help of SPSS Statistics 17.0 package (SPSS Inc., Chicago Ill). $P<0.05$ was assumed to be significant. Graphs were drawn using Microsoft Office Excel, 2010 software.

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RESULTS AND DISCUSSION

Table 2 shows the alkaloid composition of an aqueous extract of the leaves of *Acalypha wilkesiana*. Thirty nine known alkaloids were detected. The main constituents were 1.974 % echitamine, 26.226 % voacangine, 0.599 % echitamidine, 69.027 % akuamidine, 0.521 % lupanine, 0.278 % augustamine.

The effect of an aqueous extract of the leaves of *Acalypha wilkesiana* on the plasma hepatospecific markers of normal and carbon tetrachloride treated rats is given in Table 3. Compared to test control, the treatment produced significantly lower (P<0.05) plasma alkaline phosphatase, alanine transaminase and aspartate transaminase activities. The plasma total bilirubin and total bilirubin of the test groups were lower though not significantly, than that of the test control.

Table 2: Alkaloid composition of the aqueous extract of the leaves of *Acalypha wilkesiana*

Compounds	Retention time (min)	Composition (x10^{-4} mg/kg)
Choline	7.060	23.34
Trigonelline	7.530	0.69
Angustifoline	7.938	587.47
Sparteine	8.927	44.78
Ellipcine	9.745	95.73
Lupanine	11.063	1422.77
13-α-Hydrorhombifoline	11.358	77.31
9-Octadecenamide	12.828	38.75
Dihydro-oxo-demethoxyhaemanthamine	14.156	179.31
Augustamine	14.924	759.94
Oxoassoanine	15.401	107.60
Cinchonidine	16.251	195.75
Cinchonine	16.374	117.95
Crinane-3α-ol	16.459	245.13
Buphanidrine	16.674	125.33
Indicine-N-oxide	17.551	97.41
Powelline	18.593	121.73
Undulatine	18.843	90.20
Ambelline	19.690	38.81
6-Hydroxybuphanidrine	20.468	164.84
Acronycine	21.102	113.47
Monocrotaline	21.329	151.05
6-Hydroxypowelline	21.822	210.00
Nitidine	22.362	101.43
Crinamidine	23.968	590.02
1β,2β-Epoxyambelline	24.726	56.04
6-Hydroxyundulatine	24.792	69.00
Epoxy-3,7-dimethoxycrinane-11-one	25.483	21.46
Akuamidine	26.841	188641.00
Echitamidine	26.953	1638.09
Voacangine	27.065	71672.60
Mitraphylin	27.650	0.05
Camptothecin	28.194	44.95
Echitamine	28.640	5393.51
Colchicine	28.930	26.58
Emetine	29.569	10.37
Tetrandrine	29.677	5.36
Thalicarpin	30.155	5.90
Paclitaxel	32.108	1.46
Total	-	273287.00

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Table 3: Effect of an aqueous extract of the leaves of *Acalypha wilkesiana* on the plasma hepato-specific markers of normal and carbon tetrachloride treated rats

Treatment group	Liver Function Indicator				
	Alkaline phosphatase activity (U/L)	**Aspartate transaminase activity (U/L)**	**Alanine transaminase activity (U/L)**	**Total bilirubin content (µmol/L)**	**Total protein content (mg/dL)**
Normal	327.52±89.49^{a,d}	17.85±0.36^{a,d}	23.17±1.97^{a}	7.52±0.47^{a}	58.31±3.06^{a,c}
Test control	1217.16±118.33^{c}	49.88±1.67^{c}	122.72±1.02^{c}	9.10±1.25^{a}	61.91±3.57^{a,b,c}
AWC1	260.13±43.22^{d,f}	20.85±3.32^{d,f}	20.89±3.38^{a}	2.41±0.17^{c}	55.32±1.74^{c,d}
AWC2	540.04±90.35^{a,b,d,f}	20.74±2.50^{d,f}	20.57±1.58^{a}	3.24±0.19^{c}	59.55±2.91^{a,c}
AWC3	523.85±137.31^{b,f}	18.66±1.05^{d,f}	22.53±4.83^{a}	2.54±0.19^{c}	48.90±1.93^{b,d}
AW1	476.93±106.63^{a,b,d,f}	31.44±2.57^{b}	62.52±10.08^{b}	8.46±0.67^{a,b}	61.38±5.90^{a,b,c}
AW2	394.68±82.26^{a,b,d,f}	43.58±2.18^{c}	119.51±1.09^{c}	8.18±0.54^{a,b}	60.67±2.11^{a}
AW3	409.86±34.48^{a,b}	40.23±3.59^{b,c}	78.17±10.31^{b}	8.94±0.77^{a,b}	56.31±2.70^{a,b,c}

Values are mean ± s.e.m., n=5, per group
^{a,b,c} Values in the same column with different superscripts are significantly different at $P<0.05$.

The hepatoprotective activity of an aqueous extract of *Acalypha wilkesiana* leaves on carbon tetrachloride-induced hepatotoxicity in Wistar rats is shown in Figure 3. The protection against carbon tetrachloride damage seemed to be concentration dependent; with the 100 and 300 mg/kg doses being more effective. The treatment dose dependently significantly provided protection of about 83.21-92.45 % in alkaline phosphatase, 19.65-57.56 % in aspartate transaminase and 3.23-60.47 % alanine transaminase activities. The frequency distribution of the effect of an aqueous extract of the leaves of *Acalypha wilkesiana* on the liver histology of normal and carbon tetrachloride treated rats is shown in Figure 4; while sections of the liver samples are shown in Figure 5.

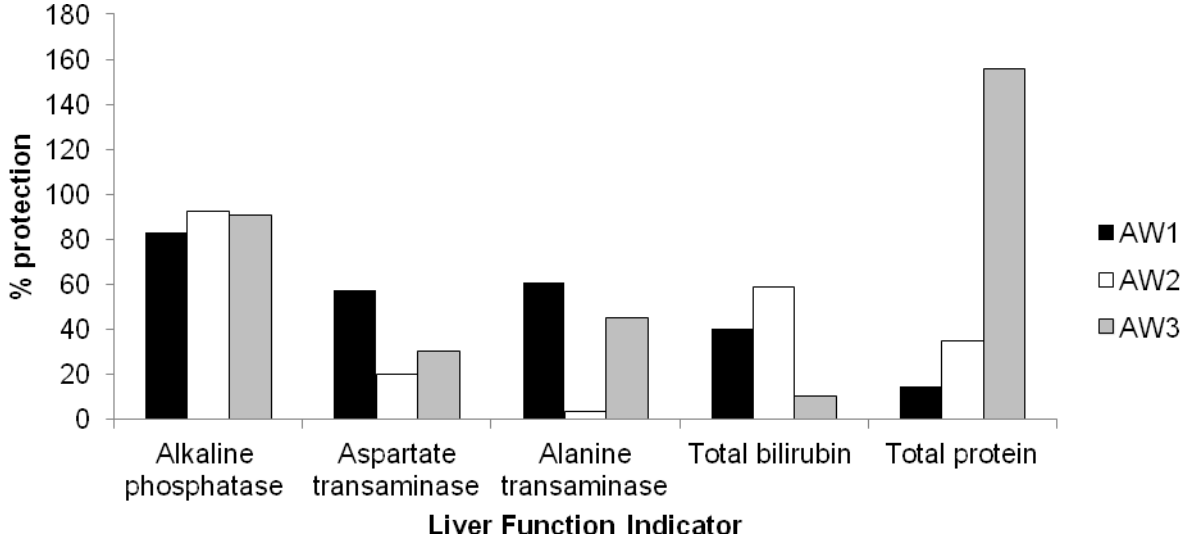

Figure 3: Hepatoprotective activity of an aqueous extract of the leaves of *Acalypha wilkesiana* on carbon tetrachloride-induced hepatotoxicity in Wistar rats

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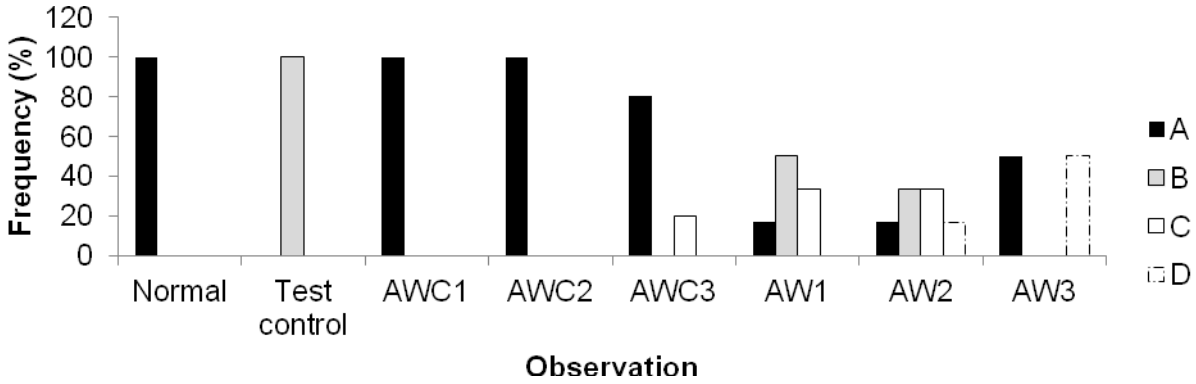

Figure 4: The frequency distribution of the effects of an aqueous extract of the leaves of *Acalypha wilkesiana* on the liver histology of normal and carbon tetrachloride treated rats. A = normal, B = fatty change, C = focal areas of fatty change, D = enlarged hepatocyte with granular cytoplasm

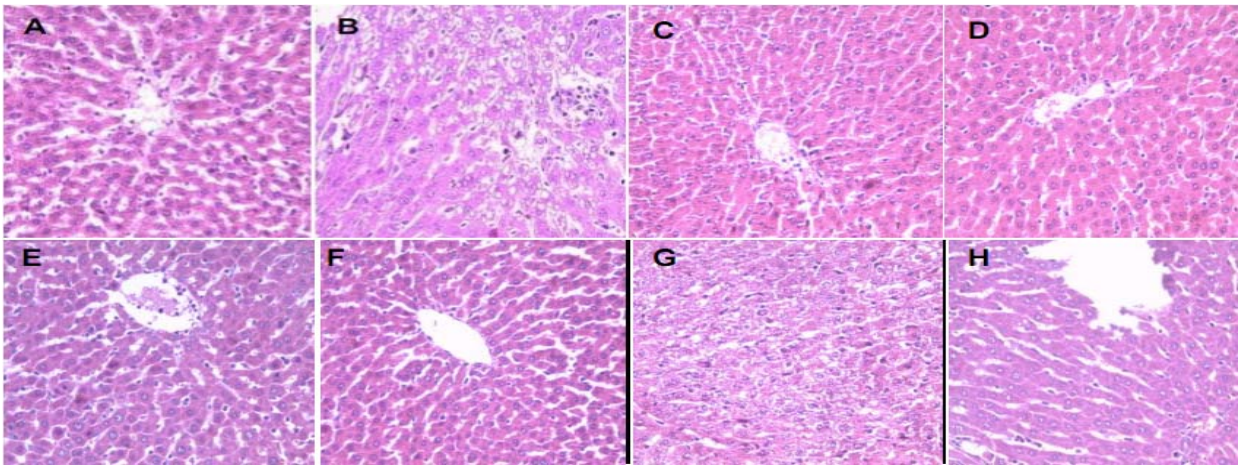

Figure 5: Sections (x20) of the liver samples showing the effect of an aqueous extract of the leaves of *Acalypha wilkesiana* on the liver histology of normal and carbon tetrachloride treated rats. A: Section of the liver of rats administered olive oil (1 mL/kg) and treated with water, showing normal cells. B: Section of the liver tissue of rats administered carbon tetrachloride (1 mL/kg) and treated with water, showing fatty change. C: Section of the liver of rats administered olive oil (1 mL/kg) and treated with 100 mg/kg extract, showing normal cells. D: Section of the liver of rats administered olive oil (1 mL/kg) and treated with 200 mg/kg extract, showing normal cells. E: Section of the liver of rats administered olive oil (1 mL/kg) and treated with 300 mg/kg extract, showing normal cells. F: Section of the liver of rats administered carbon tetrachloride (1 mL/kg) and treated with 100 mg/kg extract, with very few cells showing fatty change. G: Section of the liver of rats administered carbon tetrachloride (1 mL/kg) and treated with 200 mg/kg extract, showing normal cells. H: Section of the liver of rats administered carbon tetrachloride (1 mL/kg) and treated with 300 mg/kg extract, showing enlarged hepatocytes with granular cytoplasm.

Liver cirrhosis induced by carbon tetrachloride is perhaps the best-studied model of liver cirrhosis (Cornelius, 1993). The prevention of carbon tetrachloride-induced elevation of plasma aspartate and alanine transaminases and alkaline phosphatase activities, and plasma bilirubin level, in animals pretreated with the aqueous extract of the leaves of *Acalypha wilkesiana* shows its ability to protect normal functional status of the poisoned liver, in addition to protecting against subsequent carbon tetrachloride hepatotoxicity. The mechanism by which the extract produces its hepatoprotective activity is not certain. However, it is possible that β-sitosterol, a constituent of the extract of the leaves of *Acalypha wilkesiana* (Ikewuchi et al., 2011a), is at least partly

responsible for the protective activity against carbon tetrachloride hepatotoxicity. Earlier, Lin and Tome (1988) had reported that β-sitosterol was the anti-hepatotoxic principle in *Sambucus formosana*.

Carbon tetrachloride toxicity and its initiation of lipid peroxidation can be diminished by reducing the metabolic activation of carbon tetrachloride to trichloromethyl free radical by cytochrome P450 (Middleton et al., 2000). Therefore, any hepatoprotective agent should be able to inhibit the aromatase activity of cytochrome P450, and by so doing, favour liver regeneration. Flavonoids inhibit cytochrome P450 aromatase (Kowalska et al., 1990; Middleton et al., 2000), and may also inhibit lipid peroxidation by exerting a membrane-stabilizing action (Middleton et al., 2000). So, it can be suggested that flavonoids in *A. wilkesiana* leaves (Ikewuchi et al., 2010, 2011a), could be responsible for their hepatoprotective ability.

Earlier, Ikewuchi et al. (2010, 2011a) had reported that the leaves and aqueous extract of the leaves of *A. wilkesiana* were very rich in tannins and tannic acid. This may also be responsible for the hepatoprotective activity observed in this study. The hepatoprotective activity of tannic acid is well documented (Mittal et al., 2010; Pithayanukul et al., 2009).

Vitamin C, one of the major constituents of the leaves of *A. wilkesiana* (Ikewuchi and Ikewuchi, 2009) may also have contributed to, or be responsible for the hepatoprotection observed here. Studies have shown that hepatic microsomal drug metabolism is improved with vitamin C supplementation, probably due its augmentation of cytochrome P450 (Sato and Zannoni, 1976; Rikans et al., 1978; Burtis and Ashwood, 2001).

CONCLUSION

This study clearly demonstrates that extracts of the leaves of *Acalypha wilkesiana* are effective agents in the treatment and prevention of carbon tetrachloride-induced hepatic cytotoxicity. The data suggest that the daily oral consumption of an aqueous extract of the leaves of *Acalypha wilkesiana* was prophylactic to carbon tetrachloride poisoning.

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