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Effect of ethanolic leave extract of *phyllantus amarus* on carbon tetrachloride (CCl_4) induced hepatotoxicity in albino rats

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ABSTRACT: Liver diseases associated with its function in detoxification of xenobiotics are one of the most common causes of death. Side effects of drugs treating liver diseases have marred their popularity. This work was therefore designed to investigate the ameliorating effect of ethanolic leaves extract of *phyllantus amarus* on carbon tetrachloride (CCl_4) induced hepatotocity in albino rats. Thirty six (36) rats were divided into 9 groups (n=4rats) labeled A to I. Group A (water control) was treated with 0.2ml of distil water, B(vehicle control) received 0.2ml of olive oil, C and D were orally treated with 150 and 300mg/kgBW ethanolic leaves extract of *P. amarus* only, dissolved in olive oil for 14day respectively, 120mg/kgBW of (CCl_4) was administered to rats in groups E F,G,H and I while groups F and G , H and I orally received 150 and 300mg/kgBW of *P. amarus* leaves extract dissolved in olive oil for 7 and 14days respectively. The rats were sacrificed on days 7 and 14, blood samples were collected into heparinized sample bottles for biochemical investigation of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphate (ALP) activities, and total protein (TP), albumin (ALB) and total bilirubin (T.BIL) levels in plasma. Qualitative phytochemical screening of *P.amarus* was also carried out. Result from this work revealed significant increase ($p<0.05$) in plasma activities of AST, ALT, ALP and T.Bil level while that of TP and ALB significantly decreased ($p<0.05$) when group A was compared to group E. Non significant differences ($p>0.05$) were observed in all the parameters when group B was compared to groups C and D. All the parameters in a time and dose dependent manner, significantly decrease ($p<0.05$) except for TP and ALB levels which increased significantly ($p<0.05$) for all the extract treated groups. All the other parameters showed non significant difference ($p>0.05$) except for AST and ALP activities that were significantly increased when group A was compared to I. Result of the phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, cardenolides, steroids, tannins, carbohydrate and anthraquinones. Thus, ethanolic leaves extract of *phyllantus amarus* ameliorated the damage induced by CCl_4 on the liver. © JASEM

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Introduction

In many countries around the world plants in the genus *Phyllanthus* are used in folk remedies; therefore this genus is of great importance in traditional medicine (Foo, 1993). The genus *Phyllanthus* has a long history of use in the treatment of liver, kidney and bladder problems, diabetes and intestinal parasites. Some related species in this region with medicinal significance are *P. epiphyllanthus*, *P. niruri* *P. urinaria*, *P. acuminatus* and *P. emblica* (Tirimana, 1987). *P. amarus*, *P. nururi* and *P. urinaria* are used in the treatment for kidney/gallstones, other kidney related problems, appendix inflammation, and prostate problems (Heyde, 1990).

According to Foo and Wong (1992), in a number of countries, the aerial part of *Phyllanthus amarus* is highly valued in traditional medicine for its healing properties. This plant is traditionally used around the world in the treatment of liver ailments and kidney stones. The Spanish name 'chanca piedra' means "stone breaker or shatter stone." In South America, 'chanca piedra' has been used to eliminate gall bladder and kidney stones, and to treat gall bladder infections. *Phyllanthus amarus* has also shown to work as an antifungal, antibacterial and antiviral agent (Houghton *et al.*, 1996). Foo and Wong (1992) report that in India this plant is used in traditional medicine to treat liver diseases, asthma and bronchial infections. Chevallier (2000) notes that *P. amarus* is also used traditionally in India to treat cardiovascular problems. This popular medicinal herb is also a remedy around the world for influenza, dropsy, diabetes and jaundice (Foo, 1993). In Suriname, *P. amarus* is always sold as fresh and dry plant material in the herb markets. Heyde (1990), Sedoc (1992) and Nanden (1998) note that in traditional medicine an herbal decoction is taken to treat bladder and kidney disorders, cramps and uterus complaints (with other

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herbs). This plant decoction works also as an appetizer. *P. amarus* is a restoration herb and can be used as a tonic. In Suriname decoctions are used in herbal baths and after labor (May, 1982; Titjari, 1985; Sedoc, 1992). It is also used for colic (Wessels Boer, 1976; Heyde, 1990). According to Heyde (1990) plant extracts of *P. amarus* can be used as blood purifiers, for light malaria fevers and anaemia. *P. amarus* helps to release phlegm (Heyde, 1990), and is used to combat fever, flu (Nanden, 1998) and asthma, in combination with other herbs (Titjari, 1985). The plant, when boiled with the leaves, is considered to be a diuretic and can be used in treating diabetes, dysentery, hepatitis, menstrual disorders, and skin disorders (Heyde, 1968; Tirimana, 1987; Heyde, 1990). Sedoc (1992) notes, that in Suriname a decoction of *P. amarus* is taken along with other herbs to treat stomachache. This herb can also be used for constipation (Tjong Ayoung, 1989). Extracts from the roots can be used for jaundice. Three roots are boiled in ½ liter water and two cups are drunk daily. Decoctions for the other described ailments can be prepared by extracting two fresh plants or five dried plants in ¾ liter water drinking one cup of tea, four times per day (Heyde, 1990).

The secondary metabolites present in *Phyllanthus amarus* are alkaloids, flavanoids, hydrolysable tannins, major lignans and polyphenols. Bratati and Datta (1990) report in an evaluation study of *Phyllanthus amarus* that plant extracts have shown *in vivo* antifungal, anticancer, antispasmodic and hypoglycaemic activity. According to Thyagarajan (1988) plant extracts from this species have beneficial effects on liver functions. Mehratra et al. (1991), and Unander and Blumberg (1991) showed, using *in vitro* studies, that the *Phyllanthus amarus* extracts (polar fractions) also have antiviral activity and are a potential remedy for hepatitis B viral infection. Since the extracts of *Phyllanthus amarus* have a long history of use in tropical countries in indigenous medicine for the treatment of liver ailments, the effect of ethanolic extract of *Phyllanthus amarus* on CCl_4 induced liver damage was examined during this research.

MATERIALS AND METHODS

*Experimental Animals***:** Thirty six (36) albino wistar rats of both sexes weighing 175-200g were purchased from the Animal House of Department of Biochemistry, University of Port Harcourt, Choba Park and feed with growers feed and water *ad libitum*

*Collection and Identification of Plant***:** *Phyllantus amarus* was harvested at Choba Park, University of Port Harcourt and identified by Dr. Edwin Nwosu of Plant Science and Biotechnology Department, University of Port Harcourt with herbarium no 0609.

*Phytochemical Screening***:** Qualitative determination of the secondary metabolites was done using standard procedure as described by Sofoware (1993).

*Preparation of Plant Extract***:** The leaves of the plant were thoroughly washed and air dried for one week and shredded for blending in a warring blender to a fine powder. The powder was mixed in 95% ethanol in 1:10 ratio and allowed to stand for 48hours.Thereafter, the suspension was filtered using a Whatman no 1 filter paper and the filtrate was evaporated to remove ethanol in a water bath at 45^{o}C to obtain an oily gel like extract which was weighed and stored in a refrigerator.

Determination of LD_{50}Two doses of 150 and 300mg/kgBW of *p. amarus* extracts were the choice in this work in reference to LD_{50} determination of *phyllantus amarus* reported by Rajesh and Ramteej (2009).

*Experimental Design***:** Thirty six (36) albino rats weighing 175-200g of both sexes were divided into 8 groups (n=4rats) and kept in plastic cages. The groups were labeled A to I. Group A (water control) received 0.2ml of distil water only administered orally through an orogastric tube for 14 day. Group B (oil control) was treated with 0.2ml of olive oil orally once daily. Groups C and D (positive control) were administered orally with 150 and 300mg/kgBW oily gel of *p. amarus* extract dissolved in 0.2ml olive oil respectively using orogastric tube daily for 14 days, groups E,F,G,H and I received 120mg/kgBW of carbon tetrachloride administered intraperetoneally while groups F and G, H and I were treated orally with 150 and 300mg/kgBW of ethanolic leave *p. amarus* extracts dissolved in 0.2ml olive oil for 7 and 14days respectively to all the rats in these groups.

*Collection Of Blood And Preparation of Serum***:** The rats were withdrawn from the cages in each of the group on days 7 and 14 and placed in a desiccator containing cotton wool soaked in chloroform to partially anaesthetize the rats. The blood samples were obtained by cutting the jugular vein of the rat on the neck by means of surgical blade and put in anticoagulant sample bottle smeared with lithium-heparin . The blood samples were spun at 5000rpm using MSE centrifuge to obtain plasma.

*Chemicals and Reagents***:** All chemicals and reagents used were of analytical grade.

*Biochemical Investigation***:** Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by the method of Reitman and Frankel (1957), plasma alkaline phosphatase (ALP) activity by the method of Rec (1972), Plasma total protein and albumin were assayed by Biuret and BCG methods respectively. Determination of Bilirubin was by Jendrassik and Crod method.

*Statistical Analysis***:** Results were analyzed using (SPSS) version 15. Values are expressed in Means±Standard Deviation (M±SD).The data were expressed using descriptive statistics and Analysis Of Variance (ANOVA). Multiple comparisons for the groups were done using Post Hoc Turkey (HSD) to test for the level of significance between means. A $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Table1.Effect of ethanolic leave extract of *phylLantus amarus* on some biochemical parameters in albino rats

	Experimental groups (M±SEM)								
Parameters	Water Control Group A 0.2ml	Vehicle control GROUP B (0.2ml)	*P amarus* control for 14 days GROUP C (150mg/kg)	GROUP D (300mg/kg)	Negative control (CCl_4) GROUP E CCl_4only	(CCl_4+ *P amarus* Extract Treated for 7days GROUP F (150mg/kg)	GROUP G (300mg/kg)	(CCl_4+ *P amarus* Extract Treated for 14days GROUP H (150mg/kg)	GROUP I (300mg/kg)
AST	82.25±2.53^{bc}	136.25±3.28^{ac}	130.75±1.89^{ac}	129.75±2.02^{ac}	280.00±7.35^{ab}	192.50±3.23^{abc}	170.25±4.66^{abc}	167.66±1.03^{abc}	155.50±3.86^{abc}
ALT	29.00±0.58^{c}	35.75±1.38^{c}	31.25±1.11^{c}	31.00±1.35^{c}	44.67±2.49^{ab}	45.50±1.94^{ab}	32.00±2.20^{c}	40.00±0.82^{a}	34.00±1.35^{c}
ALP	151.25±3.90^{c}	218.75±4.27^{ac}	246.25±4.39^{ac}	248.00±1.83^{ac}	309.00±14.91^{ab}	287.25±15.53^{ab}	239.00±18.73^{ac}	259.33±3.68^{ac}	243.00±2.86^{ac}
TP	63.50±0.65^{c}	62.25±1.03^{c}	66.50±2.75^{c}	61.50±1.55	55.67±1.03^{ab}	58.50±1.19	61.50±0.65	60.34±0.62	60.50±0.65
ALB	33.50±1.04^{c}	34.50±1.26^{c}	37.50±1.85^{c}	35.25±1.03^{c}	27.33±1.25^{ab}	29.00±0.71^{b}	30.75±0.48	29.34±0.24	33.25±1.11^{c}
T.BIL	10.00±0.41^{c}	8.25±0.48^{c}	9.50±0.65^{c}	9.00±0.41^{c}	13.67±0.85^{ab}	13.00±1.30^{b}	12.25±0.63^{b}	10.00±0.41^{c}	9.00±0.41^{c}

All data are represented in Mean±Standard Error of Mean (M±SEM)
Superscript a represents significant difference($p<0.05$) when compared to water control
Superscript b represents significant difference($p<0.05$) when compared to vehicle control
Superscript c represents significant difference($p<0.05$) when compared to negative control

Table 2. Result of the phytochemical screening of ethanolic leave extract of *phyllantus amarus*

Secondary Plant Metabolites	Test	Result
Alkaloids	Drangedorfs	+ve
	Mayers	+ve
	Hagers	+ve
Flavoniods	Shinodas	ND
	Lead acetate	+ve
	Alluminium chloride	+ve
	Alkaline	+ve
Tannis	Iron (iii) Chloride	+ve
Anthraquinone	Free Anthraquinone	+ve
	Combined Anthraquinone	+ve
Triterprenoids/steroids	Lman-buchner	+ve
	Salvoski	+ve
Carbohydrate	Molsch	+ve
	Kedde	+ve
Cardenolide	Keller kilani	+ve
	Kedde	ND
Sapinins	Fronthing	+ve
	Haemolysis	ND
	Emulsion	+ve

+ve=present
-ve=absent
ND= Not Detected

Conclusion: Result from Table 1.0 indicated significant elevation ($p>0.05$) of plasma activities of ALT, AST, ALP, T.Bil and significant reduction ($p>0.05$) in TP and ALB levels when group A was compared to group E suggesting damage induced on the hepatocytes of liver tissues by CCl_4 treatment.

Marked elevation of aminotransferases in the appropriate clinical context indicates acute cell necrosis caused by the following: viral infection, drugs, toxins, alcohol, or ischemia (Burke,1975; Kamath,1996; Johnston, 1999; Dufour *et al*.,2000b). Lesser degrees of acute cell necrosis may exhibit aminotransferase values in ranges more typical of most cases of chronic cell necrosis (eg, less than seven times reference limits) (Dufour *et al*.,2000b). This variety of cell injury is the result of impaired bile flow and may be either intrahepatic or extrahepatic. In the case of extrahepatic cholestasis, both serum bilirubin and ALP are increased; the former because of a failure to excrete bilirubin, and the latter because of increased hepatic synthesis of ALP (Burke ,1975; Kamath,1996). When cholestasis is the result of focal intrahepatic disease, ALP is increased because of a cholestatic-induced increased synthesis, but bilirubin remains within normal limits. Although albumin is synthesized solely in the liver, decreased serum concentrations reflect not only liver disease but also protein loss (nephrosis, enteropathy, and burns); catabolic states; and malnutrition (Dufour *et al*.,2000a). Because of its long half-life (21 days), decreased serum albumin usually indicates chronic rather than acute disease.

Carbon tetrachloride induced liver damage has been lengthily used as an experimental model. CCl_4 is used as model drug for the study of hepatotoxicity in acute and chronic liver failure. CCl_4 is metabolized by CYP2E1, CYP2B and possibly CYP3A, to form trichloromethyl radical, CCl_3. This CCl_3 radical can bind to cellular molecules damaging crucial cellular progression. The radical can also react with oxygen to form trichloromethyperoxy radical CCl_3OO, a highly reactive species. The metabolites of CCl_4 cause the hepatic injury in CCl_4 liver injury model (Singh *et al.,* 2012). Alhassan *et al*., (2009)) concluded that high doses of the CCl_4 (90 – 120 mg/Kg) can induce massive liver damage and may persist for longer period compared to lower dosage, hence giving rise to an ideal hepatotoxicity rats model. Significants reduction($P<0.05$) and non significant increases($P>0.05$) were observed in ALT, AST, ALP, activities, T.Bil level and TP and ALB levels respectively when extract treated groups of F,G,H,and I were compared with E suggesting recovery of hepatocyte function. Table 2.0 revealed the presence of alkaloids, flavonoids, saponins, cardenolides, steroids, tannins, carbohydrate and anthraquinones. Several active compounds have been identified in *P. amarus* extract. Lignins like phyllanthin and hypophyllanthin, flavonoids like quercetin and astragalin, ellagitannins like amarinic acid and hydrolyzable tannins like phyllanthisiin D (Rajeshkumar and Kuttan,2000) isolated from this plant were reported to possess antioxidative properties. The overall recovery process and stabilization of the disease status by *P. amarus* extract might be due to its antioxidative property (Harish and Shivanandappa, 2006). *P. amarus* extract also has good antioxidant properties which help in removal of free redicals from the human body (Mhaskar *et al.,* 2000; Raphael and Kuttan, 2003; Londhe *et al.,* 2009; Harikumar *et al.,* 2009; Rai *et al.,* 2007). *Phyllanthus amarus* can detoxify the free radicals and has an antioxidant activity (Tagrajan *et al.,*1990). It has been shown to increase protein biosynthesis Tagrajan *et al.,*1998) and increase the rate of regen-eration of necrosed cells(Oudhia and Tripthi,2002. Extract of *P. amarus* exhibited protective action against carbon tetrachloride (CCl_4) induced mitochondrial dysfunction.(Padma and Setty,1999). Methanolic extract of the leaf of *Phyllanthus amarus* showed hepato protection against ethanol induced oxidative stress, (Toyin *et al.,* 2008) alloxan (Raphael *et al.,*2002) and cyclophosphamide-induced oxidative stress in rats(Kumar and Kuttan ,2005). In conclusion, *P. amarus* demonstrated ameliorating potential of carbon tetrachloride (CCl_4) induced damage to hepatocyte tissues.

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