Effect of ethanolic leave extract of *Phyllantus amarus* on carbon tetrachloride (CCl₄) 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 (CCl₄) induced hepatotoxicity 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 (CCl₄) was administrated 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 CCl₄ 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. nurari* 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
Effect of ethanolic leave extract of phyllantus amarus

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 48 hours. 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°C to obtain an oily gel like extract which was weighed and stored in a refrigerator.

Determination of LD₅₀: Two doses of 150 and 300mg/kgBW of p. amarus extracts were the choice in this work in reference to LD₅₀ 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=4 rats) 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 days. 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 14 days 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**

Table 1. Effect of ethanolic leave extract of *Phyllanthus amarus* on some biochemical parameters in albino rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental groups</th>
<th>(M±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Control</td>
<td>Vehicle control</td>
<td><em>P</em> amarus control for 14 days</td>
</tr>
<tr>
<td>Group A</td>
<td>GROUP B (0.2ml)</td>
<td>GROUP C (150mg/kg)</td>
</tr>
<tr>
<td>AST</td>
<td>82.25±2.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>136.25±3.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT</td>
<td>29.00±0.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.75±1.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALP</td>
<td>151.25±3.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>218.75±4.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TP</td>
<td>63.50±0.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.25±1.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALB</td>
<td>33.50±1.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.50±1.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T.BIL</td>
<td>10.00±0.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.25±0.48&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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 *Phyllanthus amarus*

<table>
<thead>
<tr>
<th>Secondary Plant Metabolites</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Drangedorfs</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Mayers</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Hagers</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinodas</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Lead acetate</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Allumminium chloride</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Alkaline</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>Iron (iii) Chloride</td>
<td>+ve</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>Free Anthraquinone</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Combined Anthraquinone</td>
<td>+ve</td>
</tr>
<tr>
<td>Triterprenoids/steroids</td>
<td>Lman-buchner</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Salvoski</td>
<td>+ve</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Molsch</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Kedde</td>
<td>+ve</td>
</tr>
<tr>
<td>Cardenolide</td>
<td>Keller kilani</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Kedde</td>
<td>ND</td>
</tr>
<tr>
<td>Sapins</td>
<td>Fronthing</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Haemolysis</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Emulsion</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ve=present
-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 CCl₄ treatment.

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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. CCl4 is used as model drug for the study of hepatotoxicity in acute and chronic liver failure. CCl4 is metabolized by CYP2E1, CYP2B and possibly CYP3A, to form trichloromethyl radical, CCl3. This CCl3 radical can bind to cellular molecules damaging crucial cellular progression. The radical can also react with oxygen to form trichloromethylperoxy radical CCl3OO, a highly reactive species. The metabolites of CCl4 cause the hepatic injury in CCl4 liver injury model (Singh et al., 2012). Alhassan et al., (2009)) concluded that high doses of the CCl4 (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. Significant 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 amaric acid and hydrolyzable tannins like phyllanthisin 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 radicals 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 regeneration of necrosed cells(Oudhia and Tripathi, 2002. Extract of P. amarus exhibited protective action against carbon tetrachloride (CCl4) 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 (CCl4) induced damage to hepatocyte tissues.

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Effect of ethanolic leaf extract of *Phyllanthus amarus*


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