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Effects of the Extracts of *Pycnanthus angolensis* Against Chemically Induced Acute Hepatotoxicity

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Abstract: The efficacy of the extract of *Pycnanthus Angolensis* (PA) against Carbon Tetra-chloride (CCl_4) induced hepatotoxicity was studied in rat. The study highlights information on the protective effect of PA extract as ethnomedical treatment of induced hepatotoxic injury. The dried powder of PA extract was administered orally at a dose of 300 mg/3 mL solution while liver injury was induced by carbon tetrachloride administered intraperitoneally at a dose of 0.67 mg kg^{-1} . Estimating the protein concentrations, saponins, alkaloid, glutathione and cholesterol levels monitored the hepato-protective activity. The result shows evidence of non-toxic response of PA extract against chemically induced hepatic damage. The antioxidant activities in the extract indicated its ability to protect against CCl_4 induced hepatic injury in rat. The saponins and bile acids may have interacted with cholesterol in the liver to form unabsorbed complexes, which are excreted via faeces resulting in decrease level of cholesterol in the experimental animals.

Key words: Hepatic damage, antioxidant, hypolipidaemic, cholesterol

INTRODUCTION

The exercise of toxicity of a foreign compound is a fraction of its availability at vulnerable sites. The rate of commercial introduction of new chemicals in the environment is alarming. The occupational exposure of individuals to chemical solvents for one purpose or another is a major concern because of its environmental and possible potential toxic effect and its attendant consequences vis-à-vis workforce and real-time loss hour (Rodricks, 1992). According to Rodricks, a number of occupations, including manufacturing jobs that use solvents and corrosives, can expose workers to harmful levels of chemicals.

Historically, the therapeutic trade-off of patients with hepatic problem is usually the incorporation of more chemicals in individual at risk. Rather a natural extract of a typical African Plant, *Pycnanthus angolensis* (PA) (welw) Warb family Myristiaceae (growing to a height of 20 m with a drooping branches) is widely used for ethnomedical purposes (Thomas, 1989). The crude extract of leaves and stems of the plant was shown to possess terpenoid type quinines that significantly lowered plasma glucose concentration when given orally to mice, which were hyperglycaemic (Jacob *et al.*, 2002).

In Nigeria the natives use this plant as remedy for jaundice, coated tongue and liver problems. Based on this information the present study was aimed at determining if the extract of the stem of *P. angolensis* (PA) has antioxidant effect using carbon tetrachloride induced acute hepatotoxicity model.

MATERIALS AND METHODS

Animals: Adult male albino Wister rats of both sexes were used with average weights 190 g were obtained from animal holding, Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife. The animals were randomised into three groups. One group received PA extract; another CCl_4 plus extract of PA. The third group received CCl_4 alone and the fourth served as control. All animals were found clinically healthy. The animals were kept at a room temperature ($25\pm 5^\circ\text{C}$) and humidity (relative 50%) conditions. Water was freely provided.

Extraction: The sap extract of *Pycnanthus angolensis* was obtained from the Biological Garden of the Obafemi Awolowo University, Ile-Ife. The bark of the Plant was lanced diagonally and the juice was collected over 50 mL beaker containing distilled water. After Filtration, several fractions were combined and then concentrated. The crude extract was made into powder by ROTAVAPOR-R drier and then stored in an auto-drying container until use. The powder was dissolved in normal saline to make 265 mg/3 mL solution prior to administration.

Experimental procedures: Liver injury was induced by carbon tetrachloride (CCl_4) at a dose of 0.67 mL kg^{-1} dissolved in olive oil intraperitoneally (i.p.) while the extract was administered orally by intubation. Each set comprises six animals. The first set received the extract alone; the second received the extract 1 h after CCl_4 was administered. The third set received CCl_4 alone while the

fourth received corn oil, which served as control. All animals were fasted for 24 h. before being sacrificed. The liver was removed, rinsed and homogenized in Phosphate buffer, pH 6.5. The supernatant was used for biochemical analysis.

Biochemical assay: The proximate analysis of the PA extract was determined by AOAC (1987). Saponin was measured following the methods of Ferreira *et al.* (1997) as modified by Saponin Research Update (2005). Other methods for Alkaloid (Grossell-Williams 1997), Oxidized glutathione (Hissin and Hilf, 1976) and protein concentration (Lowry *et al.*, 1951) were followed accordingly.

Statistical analysis: All data were expressed as mean±SEM and statistical significance of the data was assessed by one-way ANOVA with Scheff post-test for unpaired samples. Differences between groups were considered to be significant at $p < 0.05$.

RESULTS

The proximate analysis of the various components of *Pycnanthus angolensis* extracts is shown in Table 1. The plant aqueous extracts contents include protein and oil. The saponins test of the extract was positive in the foam

Table 1: Proximate analysis of *Pycnanthus angolensis* extract

Extract	Contents (%)
Moisture	59.4±10.15
Protein	1.1±0.04
Ash	4.9±0.17
Oil	6.23±1.50
Fibre	28.17±6.50

Values are the mean±SEM, the determination was performed in five separate extracts

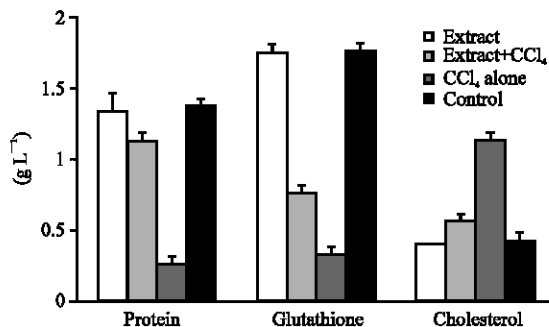


Fig. 1: Effect of PA extract on biochemical changes in experimentally induced hepatotoxicity. All values are expressed as means±SEM. Analysis of variance shows a p -value < 0.001 between the treatments for each of the biochemical changes (df 3)

and haemolytic tests (Gosmann *et al.*, 1995; Saponin Research Update, 2006). The administration of CCl_4 (0.67 mL kg^{-1}) to rats resulted in a marked decrease in protein and glutathione contents ($p < 0.5$) (Fig. 1). Prior treatment with the extracts did not have any adverse effect on protein and glutathione. However, the extracts plus CCl_4 ameliorated the alteration effect of CCl_4 . In addition, the CCl_4 alone induced significant increase in cholesterol level ($p < 0.05$), while the extract alone did not potentiate cholesterol, but rather decreased the level of cholesterol. Thus the effect of PA on the biochemical parameters is pronounced in CCl_4 -treated animals as the extract significantly reversed the changes induced by CCl_4 .

DISCUSSION

The CCl_4 -induced biochemical changes including the increased level of cholesterol as observed in this study are in agreement with previous findings (Recknagel and Glende, 1989 and Liu *et al.*, 2005). CCl_4 produces liver injury and the initial event in causing tissue damages is the conversion of CCl_4 into highly reactive intermediates such as CCl_3 radicals (Recknagel and Glende, 1989, Hostettmann and Marston, 2006). These reactive metabolites attack liver cells and other organelles, resulting in disrupted function (Liu *et al.*, 2005). This study demonstrated a significant hepatoprotective activity of PA. This assertion is based on the significant reversal of the biochemical changes produced by CCl_4 in the PA treated group and this is indicative of protection against harmful chemical toxicity.

Though saponins contents were not characterized, the PA extract may contain series of ions already known to be present in other plants (Gosmann *et al.*, 1995; Saponin Research Update, 2005). Saponins are known to form large mixed micelles with steroids (Oakenfall, 1984). Ingested saponins remain in the gastrointestinal tract and interact with cholesterol producing complexes, which prevent their absorption. The complexes with other materials (bile acids-saponins complexes) are then excreted via faeces and replenished by the metabolism of cholesterol in the liver. Thus the elimination of bile acids-saponins cholesterol complexes may result in a decrease in the cholesterol level.

The increased levels of GSH in the CCl_4 -exposed group that received PA implicate the involvement of antioxidant activity in the hepatoprotective action of the extract. This may not be surprising since free radical-mediated reactions are involved in the development of CCl_4 toxicity (Recknagel and Glende, 1989). These taken together show that the results are compatible with natural products exhibiting capacity to be hepatoprotective (Burgos *et al.*, 1997; Liu *et al.*, 2005).

Although, there is still no definitive therapy for hepatopathy, several therapeutic approaches may be used to arrest the progressive hepatic failure by correcting the biochemical abnormalities associated with it. From this point of view, many non-selective drugs are generally used clinically for treatment of hepatic injury. However, as hepatic function declines there is increasing risk of hypoglycemic episodes (Jacob *et al.*, 2002). Therefore, it is essential to search for natural drugs that are effective for treating hepatic injury without any other combined therapy and have minimal side effects, while preventing disorders of organ including liver.

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