

## EFFECTS OF *Piliostigma thonningii* ETHYL ACETATE LEAF EXTRACT ON ALUMINIUM-CUM EXTRACT TREATED WISTAR RATS

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### ABSTRACT

*Recent research findings extol the medicinal significance of the different parts of Piliostigma thonningii. The present study investigated the hepatoprotective effect of its ethyl acetate leaf extract against AlCl<sub>3</sub>-induced hepatocellular derangement in mature male rats. Thirty male Wistar rats (mean weight, 207 ± 11.01g) were randomly assigned to three groups: a control group treated with 0.5 ml of olive oil (vehicle for the extract) and 1 ml of saline (vehicle for the toxicant), a second group treated with 0.5 mg of AlCl<sub>3</sub> (toxicant) per kg of body weight (bwt) and a third group treated with 0.5 mg of AlCl<sub>3</sub> and 250 mg of P. thonningii extract per kg of body weight. Doses were administered for a period of 35 days at 24 h interval. Enzyme indices of liver functional and physiological integrity were analyzed using clinical test kits. The data obtained showed that rats treated with AlCl<sub>3</sub> expressed significant decrease in mean body weight gain (p<0.05) as well as increased serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) relative to the control group. AlCl<sub>3</sub> also caused a significant increase in the absolute weight of the liver of rats. The AlCl<sub>3</sub>-induced derangements were almost completely reversed in rats co-treated with P. thonningii ethyl acetate leaf extract. Histopathological examination of thin sections of liver of rats in the different groups showed massive hepatocellular damage in rats exposed to AlCl<sub>3</sub> alone compared to rats co-treated with the extract. Conclusively, the current study indicates that P. thonningii ethyl acetate leaf extract at the dose administered (250 mg/kg bwt po) protected rats against AlCl<sub>3</sub>-induced liver damage.*

**Keywords:** *Piliostigma thonningii*, Ethyl acetate, Aluminium chloride, Liver damage, Histopathology

### INTRODUCTION

Liver diseases are arguably the most severe ailments and major cause of death globally. Hepatotoxic agents range from chemicals (carbon tetrachloride, excess consumption of alcohol), chemotherapeutic agents (high doses of paracetamol), microbes (virus) to peroxidised oils (Maheswari *et al.*, 2008). Aluminium in its different salt forms has been reported to be hepatotoxic (Hassoun and Stohs, 1995; Chinoy and Memon, 2001; El-Demerdash, 2004). Most

of these studies reported intraperitoneal administration of this compound, which does not represent the main route of human exposure. Pulmonary and oral are the major routes through which aluminum enters the body (Testolin *et al.*, 1996). Although only a small portion of aluminum is absorbed through the gastro intestinal tract (GIT), oral intake is associated with the greatest toxicological implications (Testolin *et al.*, 1996). Nevertheless, in this study, AlCl<sub>3</sub> was administered to the experimental animals

through oral route. More than ever before, humans are continually exposed to aluminium through cooking utensils (Sharma and Mishra, 2006), food products and drinking water (Yokel and McNamara, 2001), food additives and toothpaste (Abbasali *et al.*, 2005) and through medicines such as antacids, phosphate binders, buffered aspirin, vaccines and injectable allergens (Lione, 1985; Kowalczyk *et al.*, 2004). Environmental pollution from a variety of aluminium-containing waste, especially from industrial waste water, increases human exposure beyond normal levels (Kloppel, 1997). Orthodox anti-hepatotoxic or hepatoprotective agents are expensive and unaffordable by a large size of the populace in developing countries. Although orthodox drugs are generally preferred, alternative medicine is very much relied on all over the world (O'Brien, 2004; Leckridge, 2004). According to the World Health Organization estimates, almost 80% of the people in developing countries rely entirely on traditional medicine for their primary health care and 85% of such traditional medicine involves the use of plant extract (Farnsworth, 1988).

*Piliostigma thonningii* (Figure 1) is reported to be rich in different antioxidant molecules (Aderogba *et al.*, 2004; Akindahunsi *et al.*, 2005; Ighodaro *et al.*, 2012). Many plants known to possess antioxidant properties have been proposed in the treatment and prevention of different pathologies induced by oxidative stress (Seigler, 1998). *P. thonningii* is a leguminous plant which belongs to the family, Leguminosae-Caesalpiniodae that comprises trees, shrubs or very rarely scramblers. The tree is perennial in nature and its petals are whitish to pinkish in color, produced between November and April (Jimoh and Oladeji, 2005). Its various organs: root, bark, seed, fruit, leaves, have been used for various medicinal purposes. Preliminary phytochemical studies on *P. thonningii* reveals high levels of flavonoids, tannins and alkaloids as well as nutritionally important vitamins (such as C, E and beta-carotene) all of which contributes to its strong antioxidant properties (Aderogba *et al.*, 2004; Akindahunsi *et al.*, 2005; Ighodaro, *et al.*, 2012). In many African countries *P. thonningii* is

used to treat wound, ulcers, gastric/heart pain, gingivitis and as an antipyretic. In Tanzania and Zimbabwe, a cough remedy is prepared from root bark; this fraction exhibits significant anti-inflammatory/analgesic activity in some situations (Silva, 1997). It has also been reported that certain compounds isolated from its leaves have anti-inflammatory (Silva, 1997) and antibacterial activities (Akinpelu *et al.*, 2000). The present study is designed to evaluate the possible hepatoprotective effect of *P. thonningii* ethyl acetate leaf extract on aluminium-induced hepatotoxicity in Wistar rats.

## MATERIALS AND METHODS

**Extract:** *Piliostigma thonningii* leaves were collected from the Botanical Garden, University of Agriculture, Abeokuta, southwest of Nigeria. The harvested leaves were freed of extraneous materials; air dried at room temperature and was milled into a powdery form. Five hundred grams of the powdery sample was dissolved in 2.5 litres of distilled water. The mixture was allowed to stand for 48h and stirred intermittently to facilitate extraction. The mixture was sieved using a muslin cloth. The resulting volume on sieving was reduced with a rotary evaporator at  $60 \pm 1$  °C. Final solvent elimination and drying was done using a water bath at 40 °C. A solution of the extract was prepared and appropriate aliquots were taken when required.

**Animal:** Thirty six male Wistar albino rats (mean weight,  $207 \pm 11.01$ g) used for the study were purchased from a local breeder at Oje, Ibadan, southwest of Nigeria. The animals were handled humanely, kept in metallic suspended cages in a well ventilated and hygienic rat house under standard conditions of temperature and humidity. They were maintained on normal laboratory chow (Ladokun feeds) with water *ad libitum*, and subjected to natural photoperiod of 12 hours light/12 hours dark cycle. The rats were randomly assigned to three treatment groups replicated thrice (4 rats per replicate). All animals were weighed before and at the end of the experiment.

**Experiment:** Group I (control) rats were given 0.5 ml of olive oil (vehicle for the extract) and 1ml of saline (vehicle for the extract). Group II rats were treated orally with 0.5 mg of AlCl<sub>3</sub> (toxicant) per kg bwt. Group III rats were co-treated orally with 0.5 mg of AlCl<sub>3</sub> and 250 mg/kg of *P. thonningii* extract per kg bwt. The administrations of the toxicant and the plant extract were done for a period of 35 days at 24 h interval. Animals in all the groups were given equal access to laboratory chow and water *ad libitum*.

**Biochemical Analysis:** After the last treatment, the animals were fasted overnight and weighed. Blood was collected from the retro orbital sinus of the eye by ocular puncture into non-heparinised tubes, allowed to clot at room temperature for 30 minutes and the serum was separated by centrifugation at 3000x g for 10 minutes dispensed in aliquots for biochemical analyses. The activity of alanine amino transferase (ALT: EC. 2.61.2.1) and aspartate amino transferase (AST: EC. 2.6 1.1) in blood samples were estimated by the use of end point colorimetric diagnostic kit (Randox Laboratories Limited, England) and standardized using (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) activity was determined by the use of sigma diagnostic kits (Sigma Diagnostic, USA) and standardized using (Englehardt *et al.*, 1970).

**Histopathology:** The rats were then sacrificed by cervical dislocation and the target organ, liver was quickly excised from each rat and separately washed in ice-cold 1.15% KCl solution, blotted and weighed. Parts of the liver was fixed in 10% formalin and processed for paraffin embedding using the standard micro technique. Thin sections (3µm) of the liver from rats in different groups were histologically processed, stained with Haematoxylin and Eosin and observed with light microscope for histopathological changes.

## **RESULTS AND DISCUSSION**

**Body and Organ weights:** There was a slight increase in the body weight of rats in all the

groups and the gain was lowest in rats treated with AlCl<sub>3</sub> alone. There was however no significant differences in mean weight gain among the groups except for rats treated with AlCl<sub>3</sub> (Table 1). AlCl<sub>3</sub> treatment in the current study significantly lowered the mean body weight gain of rats (by 53.7 %) when compared to the control group (Table 1). AlCl<sub>3</sub> is a heavy metal and interferes with the absorption and utilization of essential nutrients in body cells. Particularly, it competes with Mg, Zn and Ca for absorption, and as a trivalent cation (Al<sup>3+</sup>), it binds readily to negatively charged groups such as phosphate groups, nucleic acids and phosphorylated proteins. Deficiency in calcium and phosphate in the body cells affects bone formation (Szilagyi *et al.*, 1994) and reduces bone density which is an integral part of body mass. Moreover, binding of aluminium to phosphate groups severely reduces DNA and RNA synthesis (Nicholls *et al.*, 1995; Yumoto *et al.*, 2001) and inhibits protein synthesis and cell proliferation. This probably explained the significant decrease in mean body weight gain (p < 0.05) associated with rats treated with AlCl<sub>3</sub> alone. This observation corroborated with reports from earlier studies (Yousef *et al.*, 2005; Guo *et al.*, 2005) that aluminium intake results in remarkable decrease in body weights gain in rats. Kowalczyk *et al.* (2004) particularly noted significant reduction in water and food intake and transient diarrhoea, which resulted in lowering of final body mass of male rats treated with aluminium chloride for three months relative to the control group. Rats which were co-treated with *P. thonningii* extract and AlCl<sub>3</sub> were apparently protected against nutritional deficiency induced by AlCl<sub>3</sub> intake, as evident in the similar mean body weight gain of the rats (29.41g) and that of the control rats (32.25g) (Table1). This is probably due to the ability of the extract to curtail aluminium toxicity or contribute to the nutrition of the animals. AlCl<sub>3</sub> ingestion caused a marked increase in absolute weight of the liver of rats, and this change was almost completely reversed in rats co-treated with the extract (Table1). The liver is the central metabolic site of the body. Virtually all toxicants, drugs or xenobiotics are metabolized in it; this makes the organ highly susceptible to harmful



Figure 1: *Piliostigma thonningii* leaves

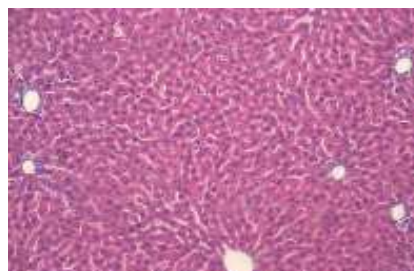


Figure 2: Tin section of liver of control rat treated olive oil and saline (vehicles for extract and toxicant showing normal histology)

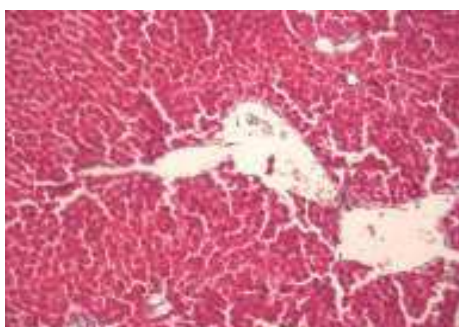


Figure 3: Tin section of liver of rats treated AlCl<sub>3</sub> showing signs of fatty degeneration, congested sinusoids and lymphocyte infiltration

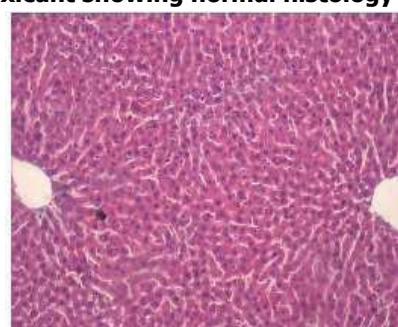


Figure 4: Tin section of liver of rats co-treated with AlCl<sub>3</sub> appearing almost as normal as that of the control rat

Table 1: Effects of AlCl<sub>3</sub> and *P. thonningii* ethyl acetate leaf extract on the body weight gain and weights of selected organs of rats

Organ	Control	AlCl <sub>3</sub> alone	EA. Extract + AlCl <sub>3</sub>
Body weight gain (g)	32.25 ± 5.30	14.92 ± 4.91*	29.41 ± 3.31**
Liver (g)	6.03 ± 1.08	6.49 ± 1.32*	5.84 ± 0.89**
Liver (g/100g bwt)	3.20 ± 1.10	3.31 ± 1.02	3.0 ± 0.56

Values are expressed as Mean ± SD (n=12). \*P < 0.05 against control, \*\*P < 0.005 against group treated with AlCl<sub>3</sub> alone. n = number of rats per group, EA=Ethyl acetate SD= Standard deviation

Table 2: Effects of AlCl<sub>3</sub> and *P. thonningii* ethyl acetate leaf extract on liver enzymes activity in serum of rats

Enzyme	Control	AlCl <sub>3</sub> alone	EA. Extract + AlCl <sub>3</sub>
ALT (U/L)	10.62 ± 2.31	18.25 ± 3.10*	12.12 ± 2.60**
AST (U/L)	13.19 ± 1.63	29.12 ± 1.90*	16.30 ± 3.42**
ALP (U/L)	119.62 ± 5.31	228.01 ± 3.52*	213.56 ± 5.81

Values are expressed as Mean ± SD (n = 12). \*P < 0.05 against control, \*\*P < 0.05 against group treated with AlCl<sub>3</sub> alone. n = number of rats per group.

metabolites generated in the process of metabolism. The increase in the weight of the liver of rats following oral ingestion of AlCl<sub>3</sub> is likely as a result of inflammation of the hepatocytes which may be associated with AlCl<sub>3</sub> metabolism or direct toxicity.

#### Liver Enzymes and Histopathology:

Compared to the control rats, AlCl<sub>3</sub> ingestion significantly (P<0.05) raised the activities of liver enzymes, ALT, AST and ALP in rats (Table 2). Elevated serum level of these enzymes is indicative of liver injury, as they are often present in large amounts in the liver and increase in the serum following hepatocellular

damage or injury. In this study, Exposure of rats to AlCl<sub>3</sub> caused significant (P<0.05) increase in ALT, AST and ALP activities by 71.8%, 120.0% and 90.6% respectively in the serum of rats treated with AlCl<sub>3</sub> as compared to the corresponding group of control rats. This is substantiated by the photomicrographs of the liver of rats in the different groups which showed high degree of fatty degeneration and lymphocyte infiltration in the liver of rats treated with AlCl<sub>3</sub> alone as compared to other groups (Figures 2, 3 and 4). Upsurge in ALT, AST and ALP activities was prevented on co-administration of *P. thonningii* extract by 33.6%, 44.02% and 6.34% respectively (Table 2). These reductions except in ALP are significant and suggest that the extract protected the rats against AlCl<sub>3</sub> mediated oxidative stress. The relatively lower decrease in serum ALP by the extract is unclear. The protective effects of *P. thonningii* could be attributed to its antioxidant properties or chelating effect on aluminium. *Piliostigma thonningii* is rich in flavonoids, tannins and alkaloids as well as nutritionally important vitamins such as C, E and beta-carotene and minerals such as calcium, magnesium, zinc and potassium, all of which contributes to its strong antioxidant properties (Aderogba *et al.*, 2004; Akinpelu, 2005; Ighodaro *et al.*, 2012).

**Conclusion:** The current study indicates that AlCl<sub>3</sub> ingestion at a dose of (0.5 mg/kg bwt po) caused decrease in body weight gain, increase in absolute weight of the liver and in hepatocellular damage in rats. Treatment with *P. thonningii* ethyl acetate leaf extract at a dose of 250 mg/kg bwt po) showed significant hepatoprotective potential against liver toxicity. This pharmacological property may be adduced to antioxidant molecules (polyphenol, vitamins and minerals) present in *P. thonningii* ethyl acetate leaf extract. We thus recommend that the required dietary allowance of aluminium in foods, water and medical drugs should be critically reviewed.

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