



Research Article

Oral Acute Toxicity Study on Methanol Extracts of *Anogeissus leiocarpus* (DC.) Guill and Perr Stem Bark in Rats

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ABSTRACT

Anogeissus leiocarpus is a herb used in African traditional medicine for treatment of many diseases. This study was aimed at evaluating the acute toxicity of methanol extract of *Anogeissus leiocarpus* (MEAL) stem bark in rats. In phase I, Nine rats were distributed into three groups of three rats each. The groups received MEAL at 10mg/kg, 100mg/kg and 1000mg/kg respectively. In phase II, Three rats were separated into three groups. Each rat received MEAL at 1600mg/kg, 2900mg/kg and 5000mg/kg respectively. All the rats were monitored for two weeks and euthanized thereafter. Serum creatinine, ALT, urea and AST levels were evaluated. The liver and kidney were processed for light microscopy. A significant decrease in urea and ALT levels were observed in rats that received MEAL at 10mg/kg and 100mg/kg relative to control. AST level was significantly elevated in rats treated with 100mg/kg and 1000mg/kg MEAL when related to the control rats. The kidneys of rats that received 5000mg/kg MEAL showed mild distortion of renal tubules. The liver of rats that received MEAL at 1600mg/kg and 2900mg/kg shows enlarged sinusoids while the liver of rats that received 5000mg/kg showed few hepatic vacuoles. Conclusively, administration of MEAL causes no mortality in rats with few signs of toxicity. It also showed normal liver and kidney functions with mild histological aberrations of the liver and kidneys at 5000mg/kg. Hence, the LD₅₀ of MEAL is above 5000mg/kg and the extract is safe.

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INTRODUCTION

Acute toxicity test estimates the adverse effects that occur after the exposure of an organism to a single dose of a substance through a particular route (oral, dermal or inhalation). The effect of any substance is related to its absorption and distribution within tissues (Saganuwan and Orinya, 2016). The LD₅₀ test (median lethal dose) was initiated by Trevan in 1927 to evaluate the dose of a substance that will result in 50% mortality in a given specie. It estimates the probable hazards of a substances in an organisms (Devi and Sangeetha, 2019). Though the main target point is death, non-lethal effects may arise as signs of toxicity. These signs depends on the amount and nature of the substance administered. These signs of toxicity may include, body weakness, erected hair, loss of appetite, diarrhoea and bulging of the eyes (Maheshwari and Shaikh, 2016).

Anogeissus leiocarpus (DC.) Guill. & Perr. is an ethno-medicinal plant in Africa and Asia (Atawodi *et al.*, 2011), it is a tall, deciduous tree with light green foliage, belonging to the family *Combretaceae* and commonly known as African birch or Axle-wood tree (Dayok *et al.*, 2018). In Nigeria, it is referred to as Marke (Hausa), Ayin (Yoruba) and Atara (Igbo) (Idakwoji *et al.*, 2019). Different parts of *Anogeissus leiocarpus* (leaf, stem bark, twigs and roots) were reported to possess antimicrobial, anti-diabetic and anti-oxidant properties and are used in the treatment and prevention of many diseases (Adefegha *et al.*, 2016). The diseases include hypertension, diabetes, liver disease and tuberculosis (Atawodi *et al.*, 2011; Victor *et al.*, 2013). The therapeutic properties *Anogeissus leiocarpus* is ascribed to the presence of alkaloids, flavonoids and tannins (Salih *et al.*, 2017).

Because of the numerous medicinal properties of *Anogeissus leiocarpus*, its use among indigenous people of Africa and Asia is increasing by the day (Lawal *et al.*, 2017). The leaf infusion is used for the treatment of cough, hay-fever and bronchitis (Mann *et al.*, 2008; Barku *et al.*, 2013). The stem bark powder is applied on gums for tooth ache. Anogelline, a substance used by the cosmetic industry is extracted from the stem bark (Shauibu *et al.*, 2008; Lawal *et al.*, 2017). In Nigeria, the roots are used as chewing sticks because they are believed to prevent mouth infection (Owoseni and Ogunnusi, 2006). The roots are also used to treat malaria and typhoid fever (Taiwo *et al.*, 1999). With the growing rate of consumption of *Anogeissus leiocarpus* due is various medicinal properties, there is need to ascertain the toxicity of the plant. Therefore, the current study aimed to determine the LD₅₀ of the methanol extract of *Anogeissus leiocarpus* (MEAL) stem bark in rats. Its effects on the liver and kidneys functions as well as their histology were also evaluated.

MATERIAL AND METHODS

Extraction

Anogeissus leiocarpus stem bark was collected from Biu, Borno state, Nigeria, it was identified by a botanist at the Department of Biological Sciences University of Maiduguri. The stem bark was air dried, grind and sieved. The powder (200g) was macerated in 70% methanol for 72 hours with constant shaking. It was then filtered and dried with a vacuum oven at 40 °C. The recovered extract was weighed.

Animal treatment

Fifteen (15) Albino Wistar rats were kept in the Animal House of the Department of Human Physiology, University of Maiduguri for 2 weeks to acclimatize. They had free access to feed (Vital Feed, Grand Cereal, Nigeria) and water. The

research was conducted following the ARRIVE Guidelines (Kilkenny *et al.*, 2011).

Experimental design

The acute oral toxicity study was carried out in accordance to the protocol described by Lorke, (1983) (Table 1). Nine rats were allocated into three groups of three rats each in the phase I. The groups received MEAL at 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. Three rats were distributed into three groups (n=1) and received 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of MEAL respectively. All the rats were monitored for two weeks for any sign of toxicity (lacrimation, salivation, tremor and diarrhoea) and/or mortality

and euthanized thereafter. Blood samples were collected, centrifuged at 5000 rpm and Serum creatinine, ALT, urea and AST levels were evaluated. The livers and kidneys were harvested, fixed in 10% formalin, and processed for light microscopy.

Statistical analysis

Data were analysed with GraphPad Prism 7. One-way analysis of variance and Tukey post-hoc test was used. Results were presented as Mean±SEM (standard error of mean) and statistical significant levels were considered at P< 0.05.

RESULTS

Rats that received MEAL at 10mg/kg, 100mg/kg and 1000mg/kg showed no physical signs of toxicity during the study period. The rats that received 1600mg/kg and 2900mg/kg MEAL

showed mild body weakness and loss of appetite while the rat that received 5000mg/kg of MEAL had erected hair, loss of appetite and difficulty in breathing. No death was recorded in all groups. Hence, the LD₅₀ of MEAL is considered to be greater than 5000mg/kg (Table 1).

Table 1: Summary of acute toxicity of methanol extract of *Anogeissus leiocarpus*

Phases	Groups	Number of Mice	Dose	Toxicity Signs	Number of death
1	1	3	10 mg/kg	None	0
	2	3	100 mg/kg	None	0
	3	3	1000 mg/kg	None	0
2	1	1	1600 mg/kg	Mild body weakness	0
	2	1	2900 mg/kg	Mild body weakness	0
	3	1	5000 mg/kg	Difficulty in breathing and bulging of eyes	0

As shown in Figure 1a, one-way ANOVA revealed a dose dependent significant increase in serum level of AST in rats administered with single dose of

MEAL at 10mg/kg, 100mg/kg and 1000mg/kg (P<0.05) respectively, when related to the control rats. However, a significant decrease in serum ALT level was observed among the rats that received 10

mg/kg and 100 mg/kg of MEAL ($P < 0.05$) compared to the control rats (Figure 1b).

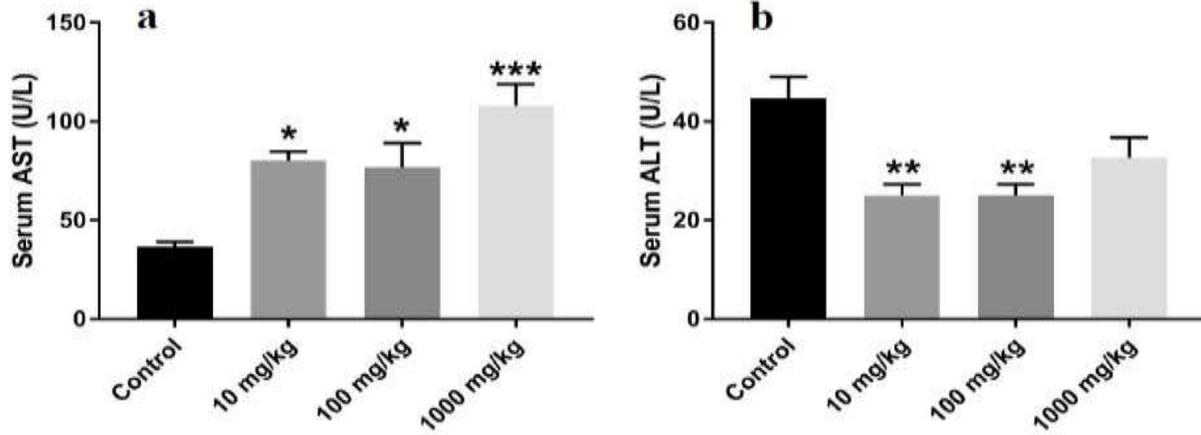


Figure 1: Serum AST and ALT levels of rats that received methanol extract of *Anogeissus leiocarpus* (n=3).

One-way ANOVA showed no statistically significant changes in the serum levels of creatinine among the rats that received 10mg/kg, 100mg/kg and 1000mg/kg of MEAL ($P > 0.05$) compared to the control (Figure 2a). Nonetheless,

there was a significant reduction ($P < 0.05$) in the serum levels of Urea in rats that received MEAL at 10mg/kg, 100mg/kg and 1000mg/kg, when compared to the control rats (Figure 2b).

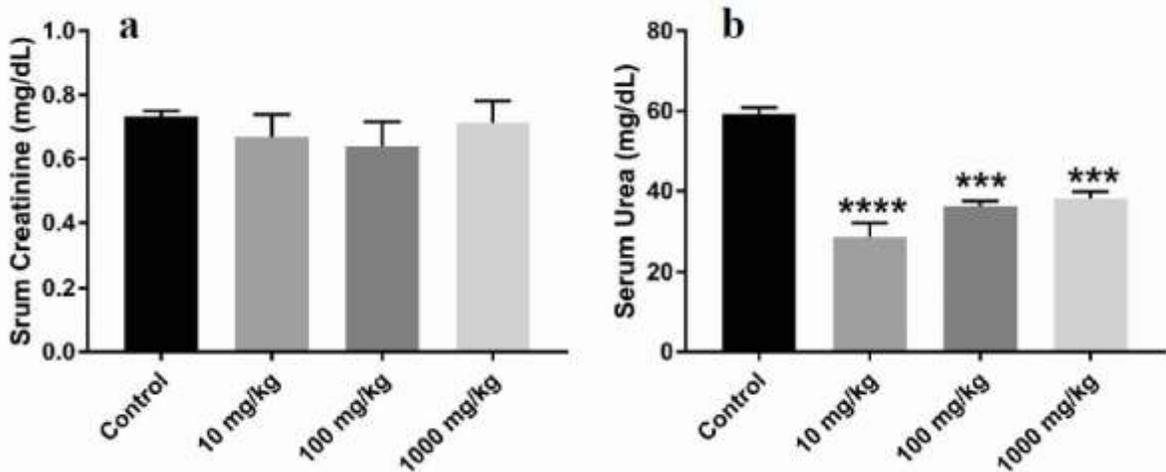


Figure 2. Serum creatinine and urea levels of rats that received methanol extract of *Anogeissus leiocarpus* (n=3).

Photomicrograph of the kidneys of control rats showed normal architecture of convoluted tubules and glomeruli (Figure 3a). Similarly, the photomicrographs of kidneys of rats from the experimental groups that received 10mg/kg,

100mg/kg, 1000mg/kg, 1600 mg/kg and 2900 mg/kg of MEAL also showed normal glomeruli and convoluted tubules (Figure 3b-f). The kidneys of rats that received 5000 mg/kg MEAL showed mild distortion of convoluted tubules (Figure 3g).

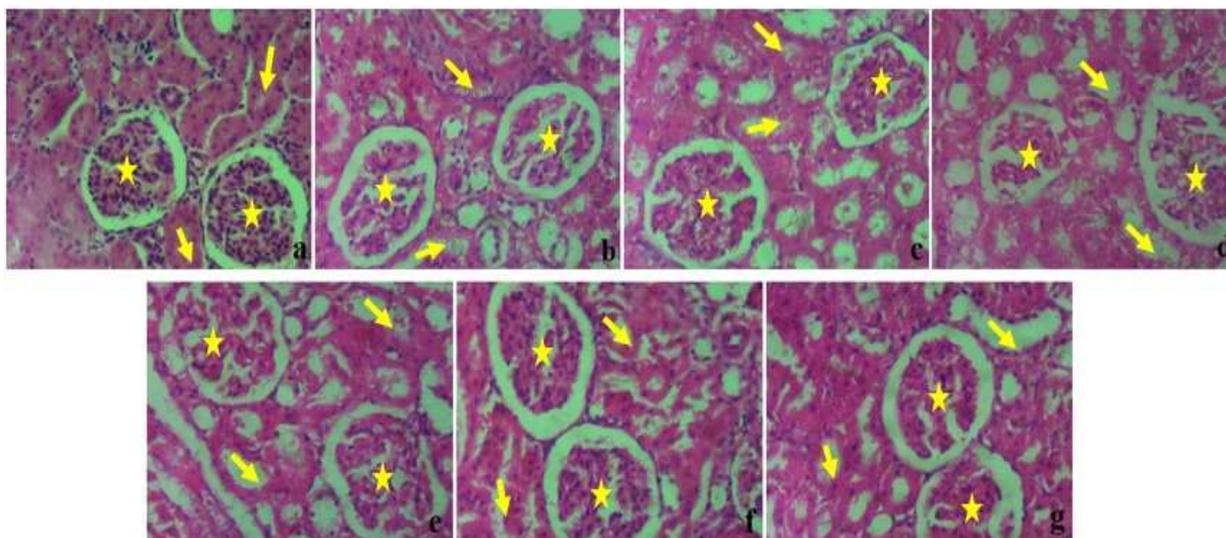


Figure 3. Kidney photomicrograph of rats that received methanol extract of *Anogeissus leiocarpus* showing glomeruli (yellow star) and renal tubules (yellow arrows). a) Control, b) 10 mg/kg, c) 100 mg/kg and d) 1000 mg/kg, e) 1600 mg/kg, f) 2900 mg/kg, g) 5000 mg/kg. Mag x200 (n=3).

Further, the photomicrograph of the liver of control group rats presented normal central vein, well arranged hepatocytes and sinusoids (Figure 4a). Consequently, the liver of the experimental rats that receive MEAL at 10mg/kg, 100mg/kg and 1000 mg/kg showed normal central vein and

hepatocytes (Figure 4b-d), while the liver of rats treated with 1600 mg/kg, 2900 mg/kg and 5000mg/kg MEAL showed normal hepatocytes with hepatic vacuole and few pyknotic cells (Figure 4e-g).

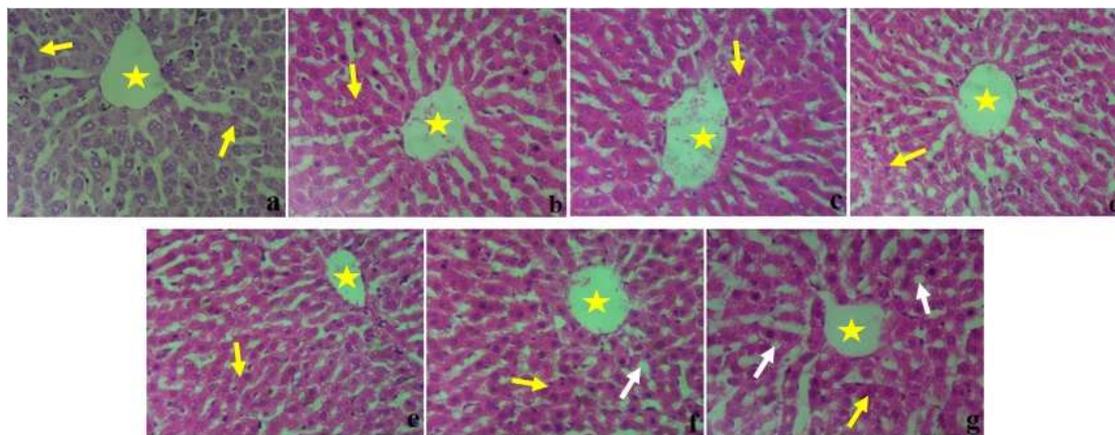


Figure 4. Liver photomicrograph of rats that received methanolic extract of *Anogeissus leiocarpus* showing central vein (yellow star), hepatocytes (yellow arrows) and hepatic vacuole (white arrows). a) Control, b) 10 mg/kg, c) 100 mg/kg and d) 1000 mg/kg, e) 1600 mg/kg, f) 2900 mg/kg, g) 5000 mg/kg. Mag x200 (n=3).

DISCUSSION

Toxicity indicates the state of deleterious effects led by the interaction between toxicants and cells (OECD, 2001; Ukwuani *et al.*, 2012). As per OECD guidelines, the first step to evaluate and fix the lethal dose for medicinal plants and its compounds is to carry out a preclinical acute toxicity study in an animal model (OECD, 2001; Teke and Kuete, 2014). The result of the present study indicates that MEAL is relatively safe for consumption because it was not lethal at 5000 mg/kg. This might be the reason for the consistence use of *Anogeissus leiocarpus* in Africa and Asia in the treatment of many diseases (Ndjonka *et al.*, 2012; Rajpoot and Singh, 2014). The OECD guidelines for classification and labelling of compounds categorised substances with $LD_{50} > 500$ mg/kg and ≤ 2000 mg/kg as no label or not classified while the global harmonized system categorise substances with $LD_{50} > 5000$ mg/kg as no label or not classified (OECD, 2001; Dibal *et al.*, 2020).

Liver failure usually causes gradual decline in liver function as a result of hepatocytes injury. This may

result in elevated AST and ALT (Lin *et al.*, 2016). The decline in serum ALT level that was observed in the present study signifies that *Anogeissus leiocarpus* had no effect on the liver. This is further confirmed by the normal liver histology that was observed in rats treated with MEAL. Following hepatic injury, ALT facilitates transfer of amino groups to form oxaloacetate (Kim *et al.*, 2008). MEAL was able to reduce oxaloacetate formation by preventing amino groups transfer. Serum AST level usually supersedes ALT level immediately after liver injury due to its high activity in the hepatocytes but with continuous injury, ALT tend to be higher because it has a longer half-life (Sheth *et al.*, 1998). The rise in AST that was observed in the present study may be due to acute muscle or liver injury that did not progress with time as confirmed by the liver histology. Earlier report showed that AST level may increase following liver or muscle injury (Wang *et al.*, 2016). AST was also reported to increase by about 2 fold after exercise (Tiidus and Ianuzzo, 1983).

The non-significant change in serum creatinine and significant decrease in serum urea in rats treated

with MEAL that was observed in the present study is an indication that MEAL does not affect kidney function. The histology of kidney also showed normal tubules indicative of normal kidney

CONCLUSION

In the present study, the cyto-architecture of both liver and kidneys remains relatively normal despite administration of MEAL. This confirmed the biochemical parameters (AST, ALT, creatinine and urea) which also remain within the

function. Increase in serum creatinine and urea are indication of kidney injury as both were reported to significantly increase in patients with renal failure compared to normal control (Nisha *et al.*, 2017).

normal range. Conclusively, the oral acute administration of MEAL at 5000mg/kg in rats produced no adverse effects during the study period. Hence, the LD₅₀ of MEAL is greater than 5000 mg/kg.

ETHICS APPROVAL

The research was approved by the Department of human Anatomy ethical committee (UM/HA/PGR19.20-08800).

COMPETING INTERESTS

None

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