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Effects of *Homalium letestui* Root Extract on Liver Enzymes, Lipid Profile, and some Haematological Indices

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Abstract
Objective: In this study, the methanol extract of *Homalium letestui* root was investigated to determine its effects on liver enzymes, lipid profile, and some haematological indices.
Methods: The crushed root of Homalium letestui was soaked in methanol for 72 h before being filtered through Whatman paper No.4 and stored at -4°C. Mature rats weighing 150-200 g were divided into four groups of six rats each. *Homalium letestui* root extract doses of 250 mg/kg, 500 mg/kg, and 750 mg/kg per body weight were administered orally daily to test groups II, III, and IV. Group I served as the control, receiving 10 ml/kg normal saline.
Results: When compared to the control, there were no significant changes ($p<0.05$-0.001) in the activities of Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in any of the test groups. The results for total and conjugated bilirubin showed an insignificant ($p<0.05$-0.001) increase in bilirubin levels when compared to the control. Furthermore, *Homalium letestui* root extract was observed to cause no significant changes in serum levels of total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL), and high density lipoprotein (HDL) cholesterol in rats at the administered doses (250,500, and 750 mg/kg bwt). When compared to the control group, treatment with the plant extract did not result in significant changes in the haematological parameters evaluated.
Conclusion: As a result, the fact that no changes were observed in the parameters assessed signifies that *Homalium letestui* has membrane stabilising and hepatoprotective properties, which may be attributed to bioactive compounds present in its root.

Keywords: *Homalium letestui*; Liver enzymes; Bilirubin; Methanol Root Extract; Haematological Indices; medicinal plant

1. Introduction

For the vast majority of the world's population, plants constitute the primary source of food and medicine. They are the primary elements in traditional healing methods and have served as inspiration for some key pharmaceutical treatments. Furthermore, medicinal plants have been utilised to treat human diseases for thousands of years. According to the World Health Organisation (WHO), medicinal plants are used by 80% of the world's population, whereas only 20% utilise pharmaceuticals prescribed by doctors [1]. Traditional therapeutic use of any plant, therefore, warrants its safety, particularly in terms of mutagenicity, nephrotoxicity, carcinogenicity, and hepatotoxicity. For example, traditional medicine makes extensive use of the herb *Homalium letestui*. It has been utilised in numerous parts of the world to treat problems such as malaria, stomach ulcers, infertility, diabetes, and so on.

Homalium letestui is a flacourtiaceae-family medicinal plant. There are approximately 60 species found in tropical Africa, with Madagascar having the most with nearly 40 species [2]. African homalium is the common name for this plant. In Nigeria, it is known as Otong Idim by the Annangs and Ibibios, Abo-Ako by the Yorubas, Akpurukwu by the Igbos, and Akporo Ekalado by the Edo people. It is a forest tree that can grow to be 80-100 feet tall and is found in West African rainforests [3]. Its bark sap is used as an enema, and its bark pulp is massaged into the skin to cure oedema. Combinations of bark decoctions are used to treat orchitis and as a tonic for mothers after childbirth. Locally, the root is utilised as an aphrodisiac and to treat malaria [2].

The plant has been reported to exhibit antiplasmodial [4], antidiabetic [5], and cellular antioxidant, anticancer, and antileishmanial [6] effects. The root extract has also been shown to have aphrodisiac properties [7]. The plant parts, particularly the root and stem bark, have long been used in various decoctions by the Ibibios of Nigeria's Niger Delta to treat stomach ulcers, malaria, and other inflammatory diseases [4]. *Homalium letestui* has been proven to possess a number of medicinally useful chemicals. The plant contains saponins, alkaloids, tannins, flavonoids, and cardiac glycosides, according to phytochemical screening [7].

Plants containing flavonoids, saponins, and tannins have been found in studies to be beneficial in the treatment of a variety of central nervous system (CNS) illnesses [8]. Sodium, potassium, phosphorus, iron, and magnesium are among the macro and micro elements found in the roots. There is a paucity of literature on the effect of *Homalium letestui* on liver enzymes, bilirubin, and haematological indices in experimental models. Thus, the current investigation is being carried out to investigate the toxicity or safety margin of *Homalium letestui* on Wistar rats' liver tissue and haematology.

2. Materials and Methods

2.1 Plant Collection

Homalium letestui roots, known as "Otong Idim" by the Annang and Ibibio people of Akwa Ibom State, Nigeria, were collected in Uyo-metropolis. A branch of the plant with leaves and flowers was presented to the University of Uyo's Department of Botany and Ecological studies for identification and authentication. A voucher specimen was kept in the Department of Botany herbarium, University of Uyo, Nigeria.

2.2 Extraction

The fresh roots of the plant were harvested from the wild, washed and drained. Subsequently, they were chopped into small pieces and air-dried for three weeks. The dried roots were pulverized to coarse powder, using mortar and pestle. The powdered root was weighed; 1kg was macerated in 99% methanol (Sigma Chemical, USA) for 72h. The liquid methanolic extract was obtained by filtration using Whatman filter paper No.4 and subsequently evaporated to dryness in a water bath regulated at 40˚C. The extract was stored at -4˚C for further experiment.

2.3 Animals

2.3.1 Determination of Median Lethal Dose (LD_{50})

The method of Miler and Tainter [9] was used to determine the median lethal dose (LD_{50}) [10]. Sixty-six healthy albino mice weighing (20-25 g) were divided into 11 groups of 6 mice per group. Different doses (200-7000 mg/kg) of the extract were administered, intraperitoneally (i.p). Physical signs of toxicity were observed, and values obtained were used to plot log probit versus concentration graph. The Median lethal dose (LD_{50}) was calculated to be 335.0±183.33mg/kg. This median lethal dose (LD_{50}) was used in this study.

2.3.2 Experimental Design

Twenty-four (24) adult wistar rats of both sexes (120-200g) and sixty (60) mice of both sexes (18-23g) were obtained from the Department of Pharmacology and Toxicology Animal House, University of Uyo, Uyo, Nigeria. The rats/mice were quarantined and acclimatized for 14 days, during which they were given free access to feed (Grower Marsh, Grand Bendel Ltd, Edo State) and water ad libitum. They were maintained under standard laboratory conditions (12 hours' light/dark cycle), and were randomly selected, identified, and kept in their cages prior to dosing. Strict care was taken to ensure that the experimental subjects were available in the appropriate size and weight range for the entire study.

2.3.3 Animal Treatment

A total of 24 rats of both sexes were divided into four groups of six rats each and treated as follows: **Group 1** was the control and was treated with distilled water (10ml/kg), while groups 2, 3 and 4 were respectively administered with 250, 500 and 750 mg/kg of the root extract of *Homalium letestui* daily for seven days. All administrations were done subcutaneously for seven days after which the animals were sacrificed under chloroform anaesthesia. Blood samples were collected through cardiac puncture using sterile needles and syringes into labelled plain sample bottles.

2.3.4 Preparation of Sample Stock

1g of the extract was dissolved in 10ml of normal saline (0.9%w/v). The mixture was allowed to stand for 15-20 min and the clear solution was decanted with the aid of a syringe and needle. The residue was dried up over a hot plate, and marc was calculated (g). The concentration of the stock solution was determined by the differences in weights between the extract used and the dried marc in 10ml of the solvent.

2.3.5 Biochemical Analysis

Blood samples were collected into each plain sample tubes (centrifuge tubes) and were centrifuged immediately at 2500 rpm for 15 min at room temperature to separate the serum. With the serum obtained, the following biochemical parameters were assayed and the determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols:

1. **Serum Albumin Assay:** Test tubes used were labelled as blank, standard, controls and samples. Reagent (1.5ml) was dispensed into each tube while 0.01 ml (10µl) of sample was added to respective tubes, mixed and allowed to stand at room temperature for 5 min. Serum albumin binds selectively to the dye bromocresol green at pH 4.2. The increase in absorbance of the resulting albumin–dye complex was read at 630nm (wavelength range: 580-630nm).
2. **Serun Total Bilirubin Estimation Assay:** Test tubes were labelled, "Blank, Standard, Control and Sample". 1ml of total bilirubin reagent was dispensed into four blank tubes. Then 1ml of the working reagent was dispensed into the labelled test tubes, and not the blank tubes. While (0.1ml) (100µl) of standard, control and sample was dispensed to its respective tube, mixed properly and left to stand for 5 min at room temperature with the wavelength set at 560nm, zeroed with blank reagent and absorbance was taken and recorded in all the tubes. Bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximum at 560 nm in the dimethyl sulfoxide (DMSO) solvent. The intensity of the colour produced is directly

proportional to the amount of total bilirubin concentration present in the sample.

3. **Serum Transaminases Assay:** Serum aspartate amino transferase (AST) otherwise known as serum glutamate oxoloacetic transaminase (SGOT) and alanine aminotransferase (ALT), also known as serum glutamate pyruvic transaminase (SGPT) activities were determined at 340nm according to the methods described by Young [11]. The activity of serum alkaline phosphatase (ALP) was determined at 405nm using standard method [12].
4. **Total Protein (Bradford Protein) Assay:** This was determined according to the method described by Bradford [13] by measuring the presence of the basic amino acid residues, arginine, lysine and histidine, which contributes to formation of the protein – dye complex. The principle of this assay is that the binding of protein molecules to Coomasie dye under acidic conditions results in a colour change from brown to blue.
5. **Lipid Profile:** Serum cholesterol, triglyceride and high density lipoprotein (HDL) levels of the experimental rats were measured using standard colorimetric methods. The low and very low-density lipoprotein (LDL and VLDL) were estimated from the formular of Friedwald *et al*. [14].

2.3.6 Haematological Analysis

Blood samples were obtained through cardiac puncture from each anesthetized/sacrificed rat using 21 gauge (21G) needles mounted on a 5ml syringe into different Ethylene Diamine Tetra-acetic Acid (EDTA) - coated sample bottles. The blood samples were analysed for red blood cells (RBC) count, hemoglobin (HGB), packed cell volume (PCV), white blood cells (WBC) and differential WBC (neutrophils, eosinophils, basophils, lymphocytes and monocytes). These parameters were analysed using automated Hematology analyser according to manufacturer's protocols at the University of Uyo Teaching Hospital, Uyo, Nigeria.

2.4 Statistical Analysis

Results were expressed as multiple comparisons of mean SEM. Significance was determined using one-way ANOVA, followed by Tukey–Kramer multiple comparison post-test. A probability level of less than 5% was considered significant.

2.5 Ethical Issues

The care and handling of animals was conducted in accordance with the National Institute of Health Guide for the Use of Laboratory Animals [15]. Moreover, Ethical approval for animal use was obtained from the Experimental Ethics Committee on Animal Use of the College of Health Sciences, University of Uyo, Uyo, Nigeria.

3. Results

3.1 Evaluation of the Effects of *Homalium letestui* Root Extract on Liver Function of Treated Rats.

The result in **Table 1** shows the effect of *Homalium letestui* root extract on liver enzymes and bilirubin. The extract caused a slim decrease in the serum levels of AST, ALT and ALP at doses 250 mg/kg, 500 mg/kg and 750 mg/kg respectively. The decrease was statistically significant ($p<0.05$) at 500 mg/kg for AST. ALT showed significant decreases at 250 mg/kg and 500 mg/kg ($p<0.001$). The extract also had a non-significant decrease in total bilirubin. However, there was an increase in the level of combined bilirubin which was also not significant statistically when compared to normal control.

Table 1: Effect of Extract on Liver Enzymes and Bilirubin of Treated Rats

Dose (mg/kg)	AST (1µ/L)	ALT (1µ/L)	ALP (1µ/L)	TB (µmol/L)	CB (µmol/L)
CT	91.67±5.77	36.83±1.70	40.67±2.14	3.08±0.12	1.85±0.12
250	71.17 ± 5.75^{ns}	26.83 ± 1.25^{c}	30.17 ± 1.14^{b}	3.05 ± 0.11^{ns}	2.03 ± 0.09^{ns}
500	69.67 ± 5.74^{a}	24.17 ± 0.79^{c}	33.67 ± 1.75^{ns}	3.05 ± 0.13^{ns}	2.08 ± 0.21^{ns}
750	100.2 ± 3.45^{ns}	36.50 ± 1.73^{ns}	39.67 ± 2.03^{ns}	3.15 ± 0.14^{ns}	2.00 ± 0.10^{ns}

Values represent Mean ± S.E.M; Significance relative to control: $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.
(n=6); ns= not significant; TB= Total Bilirubin, CB= Conjugated Bilirubin, Test.= Testosterone, CT= control.

3.2 Effect of Extract on Lipid Profile of Treated Rats

The root extract of *Homalium letestui* was observed to cause slight and insignificant reduction in the levels of total cholesterol, triglyceride and very low density lipoprotein as seen in Table 2. However, at dose 750mg/kg, there was slight increase that was statistically not significant. The extract also caused a non - significant increase in the levels of high density lipoprotein and low density lipoprotein when compared to normal control.

Table 2: Effect of Extract on Lipid Profile of Treated Rats

Dose (mg/kg)	TC (mmol/L)	TG (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	VLDL (mmol/L)
CT	2.43±0.15	1.65±0.07	1.05±0.10	0.68±0.04	0.70±0.04
250	2.73±0.11	1.58±0.03	1.23±0.06	0.83±0.06	0.67±0.02
500	2.27±0.16	1.57±0.03	1.00±0.11	0.63±0.06	0.65±0.02
750	2.97±0.33	1.62±0.09	1.43±0.21	0.80±0.12	0.73±0.04

Values represent Mean ± S.E.M; Significance relative to control: $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.
(n=6); ns= not significant. TB= Total Bilirubin, CB= Conjugated Bilirubin, Test.= Testosterone, CT= control.

3.3 Effects of Extract on Some Haematological Parameters of Treated Male Rats

The administration of the methanol root extract of *Homalium letestui* caused insignificant decreases in RBC, WBC, HGB, MCV, MCH, lymphocytes and monocytes when compared to control (Table 3). The extract also caused a decrease in the percentage of lymphocytes which was statistically significant ($p < 0.01$- $p < 0.001$); the decrease was not dose-dependent. The platelet counts increased significantly ($p< 0.01$) in the groups treated with 250mg/kg but had slight increases in the other pretreated groups (Table 3).

Table 3: Effects of Extract on Haematological Parameters of Treated Rats

Dose (mg/kg)	WBC (×10^{9}cells/L)	RBC (µL×10$^{6)}$	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	PLT (×10^{9}cell/L)	LYM (%)	MCHC (g/dl)
NS	14.967±1.55	7.51±0.57	13.77±0.35	40.33±2.87	53.88±0.70	18.96±1.87	354.2±42.88	88.73±1.40	34.98±2.47
250	10.27±1.40^{ns}	7.49±0.45^{ns}	13.40±0.78^{ns}	35.76±2.27^{ns}	47.68±0.62^{ns}	17.86±0.27^{ns}	835.4±31.71*	66.92±1.05^{a}	37.52±0.29^{ns}
500	11.06±1.97^{ns}	7.76±0.14^{ns}	13.63±0.26^{ns}	36.32±0.89^{ns}	47.25±0.48^{ns}	17.58±0.19^{ns}	584.33±28.27^{ns}	67.07±2.64^{a}	37.35±0.09^{ns}
750	9.54±0.52^{ns}	7.40±0.22^{ns}	13.42±0.44^{ns}	36.35±1.20^{ns}	48.88±0.84^{ns}	18.23±0.23^{ns}	586.67±42.33^{ns}	61.48±4.24^{b}	37.33±0.35^{ns}

Values represent Mean ± S.E.M; Significance relative to control: $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.
(n=6); ns= not significant; WBC= White blood count, RBC= Red blood count, HGB= Heamoglobin, MCV= Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, LYM= Lymphocyte, PLT=Platelet count, MCHC= Mean corpuscular haemoglobin concentration

4. Discussion

The effect of *Homalium letestui* methanol extract on biochemical parameters and haematological indices of treated animals was investigated in this study. To investigate the toxicological effect of this plant, liver function tests, lipid profiles, and haematological analyses were performed. Liver enzymes are well-established biomarkers for predicting liver toxicity [16]. The activities of serum AST, ALP, ALT, bilirubin (total and conjugated), and total cholesterol can be utilised to assess liver function [17]. AST and ALP are abundant in the liver, where they participate in amino acid metabolism [18].

ALP, on the other hand, is predominantly found in the bile duct of the liver and can be utilised to assess biliary function, cholestasis, and hepatic function [18]. When the liver is diseased, these enzymes leak into the bloodstream and act as an indicator of liver impairment [19]. The observed non-significant decreases in AST and ALT activity in all test groups when compared to the control may indicate that the extract has a hepatoprotective effect. A slight decrease in ALP serum levels suggests that the extract may have hepatocyte-protective properties. The decrease in serum enzymes in the treated groups could be attributed to the extract preventing intracellular enzyme leakage. The findings of this study corroborate those of Okokon *et al.* [20], who reported on the extract's hepatoprotective properties. Similarly, Aquaisua *et al.* [21] observed that crude extracts of Blighia unijugate, for example, have no harmful effects on the kidney or liver of rats [22]. This supports the notion that some plant extracts may have hepatoprotective properties, whilst others, such as Vitex doniana and Sorghum bicolor, have toxic effects on the kidney and liver [23].

Increased serum ALP levels are caused by increased synthesis in the presence of increasing biliary pressure [24] and reflect pathological changes in biliary flow [25]. There was no increase in serum ALP in any of the treatment groups in this investigation, indicating that the extract had no detrimental effect on biliary flow. This supports Okokon *et al.* [20]'s investigation on the extract's membrane stabilising action. Bilirubin is a haemoglobin metabolic product that is conjugated with glucuronic acid in hepatocytes to increase its water solubility. Its diagnostic significance is an index for assessing hepatic function, necrosis severity, conjugation, and excretory capacity of hepatocytes. Elevated levels of total bilirubin are produced by liver cell dysfunction and result from decreased uptake and conjugation of bilirubin, whereas elevated levels of direct or conjugated bilirubin are caused by decreased secretion from the liver or obstruction of the bile ducts [26].

However, this study found a negligible rise in total and conjugated bilirubin levels when compared to the control group. The small increase in serum levels of total and conjugated bilirubin detected is nonetheless evidence that the extract had no deleterious effect on the liver's normal functioning status [7]. Lipids are often insoluble in aqueous or polar solvents but highly soluble in nonpolar or organic solvents. Aqueous medium is commonly used for biochemical processes and molecular transport. As a result, lipids are normally combined with certain proteins to create structures known as lipoproteins, which have a high degree of hydrophilicity. Low density lipoproteins, high density lipoproteins, and chylomicrons, which are primarily constituted of triglycerides, are all components of serum lipoproteins [27]. Except for HDL cholesterol, high levels of all lipids in the blood are arguably a significant risk factor in the onset of cardiovascular diseases. High triglyceride and LDL levels in the blood have been linked to atherosclerosis and coronary heart disease [28]. Cholesterol is the most abundant sterol in animal tissues and is found primarily in cell membranes due to its amphipatic nature [29]. It is also found in the adrenal gland, liver, brain, and nervous system [30]. The molecule is primarily synthesised in the liver from acetyl CoA, after which it is transferred through the blood to extrahepatic tissues where it is used for the synthesis of bile acids and steroid hormones, as well as the regulation of membrane fluidity. However, high levels of cholesterol in the blood have negative consequences on human health. It is said to be a primary cause of cardiovascular disorders such as atherosclerosis, myocardial infarction, and coronary heart disease.

In this investigation, *Homalium letestui* root extract at the administered doses (250,500, and 750 mg/kg bwt) was found to have no effect on serum levels of total cholesterol, triglycerides, LDL, VLDL, and HDL cholesterol in rats. This could imply that the extract has a preventive role against degenerative diseases. Furthermore, the phytochemicals found in medicinal plants are primarily responsible for the distinct pharmacological effects they have on the human body. Flavonoids, alkaloids, cardiac glycosides, and tannins have been shown to play critical roles in lipid metabolism as well as preventive actions against lipid peroxidation and cardiovascular disease [31].

Homalium letestui contains a high concentration of flavonoids, tannins, and alkaloids [32]. These chemicals are most likely responsible for the plant's hypolipidaemic activity observed in this study. The critical role of blood cells, along with this highly proliferating tissue's susceptibility to xenobiotic intoxication, makes the hematopoietic system a unique target organ [33]. The various blood components that are analysed to gauge an individual's health and sickness state are referred to as haematological parameters. Red blood cells (RBCs), white blood cells (WBCs), platelets, haemoglobin, haematocrit, and several other indications are among these measures. Haematological parameters are important in the diagnosis and treatment of a wide range of diseases, including anaemia, infections, bleeding disorders, and cancer.

A study published in the journal Clinical and Diagnostic research found that haematological characteristics play a significant role in diagnosing and monitoring haematological disorders such as anaemia, leukaemia, and thrombocytopenia [34]. Another study published in the journal of Medical Sciences emphasises the significance of haematological parameters in the diagnosis and management of sepsis, a potentially lethal infection-related condition [35]. Assessment of haematological parameters is a good tool for determining an individual's health status and assessing the harmful impacts of toxic compounds [36]. Changes in the haematological system can help predict toxicity in animals (Olson *et al*., 2000), and changes in red blood cell (RBC), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) concentrations are particularly important for diagnosing anaemia. These parameters reveal not only the potential dangers of herbal remedies, but also their impact on blood-related disorders. When compared to the control group, treatment with the plant extract did not result in significant changes in RBC and HGB levels (Table 3). This demonstrates that the extract is unlikely to produce haematological problems in humans, such as bleeding, anaemia, or bone marrow suppression. This study supports the findings of Arsad *et al*. [37], who found that the plant extract was rather safe in terms of haematological effects. Table 3 shows that the white blood cell (WBC) count decreased slightly but not statistically significantly.

The decline was shown to be dose-dependent. WBCs and their variations, such as lymphocytes, serve as indicators of the body's reaction to potentially dangerous substances, including those derived from plants [38]. WBCs and lymphocytes are important components of the body's defence mechanism. WBC counts were within the normal physiological range in all treatment groups. The lymphocyte percentage values in the treatment groups were within the normal physiological range of 65.00-84.50% (Table 3). Furthermore, as shown in Table 3, as compared to the control group, all groups treated with the extract had a non-significant decrease in RDW-SD (red blood cell distribution width-standard deviation). Despite the fact that this parameter is commonly reported as part of a complete blood count and is primarily used to narrow the differential diagnosis of anaemia, there is a definite relationship between RDW and the likelihood of bad outcomes in heart failure [39].

5. Conclusion

Overall, the methanol root extract of *Homalium letestui*, a plant used by the Annangs and Ibibios of Nigeria's Niger Delta to treat stomach ulcers, malaria, and other inflammatory diseases, as well as an aphrodisiac (Okokon *et al*., 2006; Okokon *et al*., 2007), has no discernible adverse effects on liver function and haematological indices in wistar rats. Long-term consumption of the plant, on the other hand, should be avoided because no research has been undertaken to determine its long-term use.

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7. Competing interests

Authors have declared that no competing interests exist.

8. Authors' Contributions

The authors worked together to complete this work. The final manuscript was read and approved by the authors.

9. Ethical Approval

Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo, Nigeria.

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