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# Hematological and biochemical indices on subchronic administration of *Setaria megaphylla* (Steud) T. Dur and sphinx (Poaceae) root extract on rats

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**Abstract**
**Background***: Setaria megaphylla* (Steud) T. Dur and Schinz (Poaceae), is a medicinal plant popularly used South-South Nigeria to treat malaria, hemorrhoids, urethritis, inflammation, diabetes, fevers and various pains(Udobang, Okokon and Etuk, 2016) [1]. The persistent and consistent use of *Setaria megaphylla* by the locals to treat various ailments therefore necessitated this investigation of its effects on blood.
**Objectives**: This work was therefore designed to investigate the effects of *Setaria megaphylla* ethanol root extract on hematological and biochemical parameters during sub chronic toxicity administration.
**Methodology**: The effects of *Setaria megaphylla* ethanol root extract (150, 300, 450 mg/kg) on hematological and biochemical indices (liver and kidney functions) in rats were investigated for using standard procedures.
**Results**: There was non-significant effects on the hematological and biochemical parameters during sub chronic toxicity investigation.
**Conclusions**: The findings of this study revealed that *S. megaphylla* ethanol root extract exhibited negligible toxic effects.

**Keywords:** Hematological, biochemical *Setaria megaphylla*, root extract, medicinal plant

## 1. Introduction
*Setaria megaphylla* is a perennial broad-leafed bristle grass, with robust roots 30 cm diameter at the base (Bromilow, 1995) [2]. Its leaves are soft, bluish grey green in colour, 1 m long and 10 cm broad. Its edges are glabrous and scarbrid with compressed and more or less keeled leaf sheaths (Bromilow, 1995) [2]. It is usually found besides rivers, in low lying areas or forests and in areas with a lot of moisture, like tropical and subtropical areas of Africa and America (Van Oudtshoorn, 1999) [3].
A leaf-decoction is sedative on cough, and also indicated for oedema (Burkill, 1985)[4]. Ijo in South-East Nigeria rub leaves crushed with salt on the forehead for headache, and squeeze the sap on to a sore after it has been cleaned. The grass has a reputation for beneficial action on urino-genital troubles. Pressed juice of *Setaria megaphylla* leaves are used to treat anuria. The plant has anodynal and analgesic properties. Zulus in South Africa apply crushed leaves to bruises. In Republic of the Congo, sap is massaged into areas of pain. For more vigorous action the affected part may be scarified by rubbing with the rough leaf, and ash of the calcined plant applied (Burkill, 1985) [4].

## 2. Materials
### 2.1 Collection and Identification of Plant Sample
The roots of *Setaria megaphylla* were collected from Anwa forest in Uruan Local Government area of Akwa Ibom State, Nigeria. They were identified and authenticated in the Department of Botany and Ecological Studies, University of Uyo, Uyo. A voucher specimen (FPHUU 221) was deposited in the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

### 2.2 Animal Stock
Adult albino rats and mice were obtained from the Animal House of the University of Uyo, Uyo, Akwa Ibom State and were maintained in the University of Uyo Animal House and fed

with growers pellet feed (Bendel Feeds and Flour mills Ltd, Edo State) with water given *ad libitum.*

## 3. Methods

### 3.1 Extraction

The roots were washed and dried under shade until a constant weight was gotten. The dried roots were cut into small pieces and grinded into powder using a blender. The powdered roots was macerated in 70 % ethanol for 72 hours, and the liquid filtrate concentrated and evaporated to dryness in a water bath at 60 °C. The yield was then weighed and stored in a refrigerator at -4 °C until used for the proposed experiments.

### 3.2 Toxicological Studies

Acute toxicity studies were carried out to determine the median lethal dose ($LD_{50}$) using the Miller and Tainter (1944) [5] method as reported by Udobang, Okokon and Ettebong (2017). The mice were treated with various doses (1000 - 5000 mg) of the ethanol extract and observed for 24 hours. Physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and deaths were recorded.

Subchronic studies were carried out with emphasis on the effect of the extract on liver and kidney functions and the on hematological and biochemical indices.

### 3.3 Evaluation of Subchronic Toxic Effect of Extract in Rats

Adult albino rats were weighed and randomized into four groups of six animals each. Group 1 received 10 ml/kg of distilled water orally, and served as control. Groups 2, 3 and 4 received the ethanol extract at 150, 300 and 450 mg/kg orally respectively. Drugs were administered daily for 28 days at 09.00 hours. Mortality was monitored daily and weight changes of animals was recorded weekly. On day 29, after an overnight fast, the animals were anaesthetized with light chloroform and blood samples collected by cardiac puncture for hematological and biochemical analyses into tubes with or without EDTA respectively. Haemoglobin (Hb) (g/dl), packed cell volume (PCV) (%), red blood cell count (RBC) (µL), total and differential white blood cell count (WBC) (µL) and platelet count (µL) were determined using automatic counter Sysmex (K 21, Tokyo, Japan).

The biochemical parameters were determined in serum obtained after centrifugation of total blood without anticoagulant, at 2500 rpm for 15 min. Standardized diagnostic kits and spectrophotometer were used for spectrophotometric determination of the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Others were creatinine, urea, total protein, albumin, $Na^+$, $K^+$, $Cl^-$ and $HCO_3^-$, total and direct (conjugated) bilirubin and total cholesterol.

### 3.4 Statistical Analysis

The data was analysed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison post-test. Differences between means were considered significant at 5 % and 1 % level of significance ie $p \leq 0.05$ and 0.01.

## 4.0 Results of Pharmacological Investigations

### 4.1 Effect of extract on hematological indices

On the hematological indices, the extract (150 - 450 mg/kg) produced a non-significant effect on RBC, PCV, Hb, WBC, lymphocytes, basophils, eosinophils, monocytes and neutrophils in the low and middle doses. The 300 – 450 mg/kg extract showed a significant ($p < 0.05$ - 0.01) reduction in platelets while the 150 mg/kg caused a significant ($p < 0.01$) increase. There was a significant ($p < 0.05$) increase in neutrophils in the high dose of the extract (Table 1).

### 4.2 Effect of extract on clotting time

The effect of the extract on the clotting time was non-significant and non-dose-dependent during the sub chronic investigations (Table 2).

### 4.3 Effect of extract on liver function:

The extract (150 – 450 mg/kg) caused a non-significant increase in the weight of liver, AST, conjugated bilirubin and albumin and a non-significant reduction in total cholesterol in the sub chronic investigations. While there was no significant effect on levels of total protein, albumin and total bilirubin, there was however a significant ($p < 0.01$) increase of ALT and of ALP ($p < 0.05$) by the extract. (Table 3).

### 4.4 Effect of extract on kidney function:

The extract (150 – 450 mg/kg) produced no significant effect on the levels of $Na^+$, $K^+$, $HCO_3^-$, $CL^-$ and creatinine. However there was a significant ($p < 0.001$) increase in level of urea (Table 4).

**Table 1:** Effect of extract on hematological indices of rats after subchronic administration

| Treatment/ dose(mg/kg) | RBC ($X10^6$/µL) | PCV (%) | WBC (fl) | NEUT (pg) | Lymphocytes (g/dl) | Hb (g/dl) | Platelets $x10^3$/ µL | Monocytes | Eosinophil | Basophil |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 4.68±0.15 | 48.20±1.15 | 5.46±0.55 | 17.97±0.19 | 77.4±2.18 | 16.08±0.37 | 158.2±2.63 | 1.60±0.81 | 0.00±0.00 | 0.20±0.26 |
| Extract 150 | 4.46±0.25 | 35.87±0.19 | 4.82±0.66 | 18.13±0.42 | 79.8±1.68 | 15.66±0.26 | 177.2±2.23[b] | 0.60±0.24 | 1.20±0.77 | 0.00±0.00 |
| Extract 300 | 4.62±0.20 | 38.43±0.20 | 4.62±0.20 | 18.10±0.00 | 77.8±2.28 | 15.72±0.43 | 124.4±15.61[b] | 1.02±0.44 | 0.40±0.40 | 0.00±0.00 |
| Extract 450 | 4.92±0.17 | 37.43±0.19 | 5.76±1.09 | 19.03±0.1[a] | 73.8±1.46 | 17.4±0.61 | 111.2±5.42[a] | 3.20±1.02 | 0.4±0.40 | 0.40±0.24 |

Data are expressed as mean ± SEM. significant at [a]$p < 0.05$, [b]$p < 0.01$, when compared to control, n = 6.

**Table 2:** Effect of ethanol extract on clotting time

| Treatment(mg/kg) | Clotting time (mins) |
|---|---|
| Distilled water | 0.45 ± 0.01 |
| Extract 150 | 1.81 ± 0.18 |
| Extract 300 | 0.46 ± 0.03 |
| Extract 450 | 0.70 ± 0.16 |

Data are expressed as mean ± SEM, n = 6.

**Table 3:** Effect of extract on liver function of rats after sub chronic administration

| Treatment mg/kg | Total Protein (g/dl) | Albumin (g/dl) | Total Bilirubin (mg/dl) | Conjugated Bilirubin (mg/dl) | AST (iu/l) | ALT (iu/l) | ALP (iu/l) | Total Cholesterol mmol/l | Liver weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 6.62± 0.24 | 4.06±0.21 | 18.2±0.66 | 10.6±0.67 | 86.0±7.87 | 36.2±1.74 | 100.4±8.25 | 5.20± 0.62 | 6.53±0.23 |
| Extract 150 | 6.15±0.28 | 4.08±0.87 | 16.8±0.58 | 10.8±0.37 | 111.4±2.53 | 48.4±2.54[b] | 133.2±14.46 | 2.56±0.10 | 7.63±0.20 |
| Extract 300 | 6.00±0.20 | 3.97±0.68 | 18.6±0.81 | 10.8±0.96 | 103.6±2.42 | 42.6±2.63 | 149.8±9.23[a] | 2.40±0.08 | 6.76±0.14 |
| Extract 450 | 6.16±0.30 | 4.10±0.92 | 19.0±0.54 | 10.8±0.37 | 107.0±0.89 | 48.8±1.31[b] | 164.6±14.06 | 3.82±0.60 | 6.62±0.15 |

Data were expressed as mean ± SEM. significant at [a]$p < 0.05$, [b]$p < 0.01$, when compared to control, n = 6.

**Table 4:** Effect of extract on some kidney function parameters of rats after sub chronic administration

| Treatment (mg/kg) | $Na^+$ (mmol/l) | $K^+$ (mmol/l) | $CL^-$ (mmol/l) | $HCO^-_3$ (mmol/l) | UREA (mmol/l) | Creatinine (mmol/l) |
|---|---|---|---|---|---|---|
| Control | 164.2±6.53 | 8.44±0.58 | 117.6±4.00 | 19.4±0.40 | 3.64±0.18 | 102.6±6.89 |
| Extract 150 | 156.0±3.05 | 8.40±0.50 | 113.6±1.91 | 19.0±0.00 | 5.92±0.29[c] | 106.0±5.56 |
| Extract 300 | 154.4± 3.90 | 7.48±0.21 | 110.8±2.70 | 18.6±0.73 | 4.20±0.28 | 100.4±7.01 |
| Extract 450 | 164.2± 6.53 | 7.70±0.84 | 115.8±2.72 | 18.8±0.73 | 4.40±0.31 | 98.6±6.74 |

Data are expressed as mean ± SEM. significant at [c]$p < 0.001$ when compared to control, n = 6

## 5. Discussion

In the sub chronic toxicity investigations, there was no significant increase in the weight of the animals or the organs. This was corroborated by a non-significant effect in the level of total proteins and albumin. Increase in body weight of animals is usually attributed to the presence of active metabolites such as saponins and alkaloids (Joo, Cho and Kwon, 1978; Eteng, Etarrh and Owu, 2003) [10, 11] which though present in this extract were not identified in the most active fractions (Udobang, Bassey and Okokon, 2017) [6, 12].

RBCs and related factors are major indices used to evaluate circulatory erythrocytes, diagnose anemia and also serve as useful indices of the bone marrow capacity to produce RBCs in mammals (Ozkan, Kaya and Akgul, 2012) [13]. A significant increase in RBC, PCV, Hb and MCHC is usually an indication of erythropoiesis stimulation of an extract which must have increased the rate of erythropoietin release in the kidney, which is the humoral regulator of RBC production (Mishra and Tandon, 2012) [14]. There was no significant effect on the levels of red blood cells (RBC), packed cell volume (PCV) and hemoglobin (Hb) indicating that the extract did not cause anemia.

The major functions of the white blood cells (WBCs) and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies (Lawal *et al*, 2015a) [15]. A significant increase in the WBC and lymphocytic count by a plant extract reflects leucopoetic and possible immunomodulatory effects (Bashir *et al*, 2015) [16], which will enhance an animal's capability to generate antibodies during phagocytosis and have high degree of resistance to diseases with enhanced adaptability to local environmental and disease prevalent conditions (Okunlola, Olorunisomo, Aderinola, Agboola and Omole, 2012) [17]. There was no significant effect on WBC, lymphocytes, basophils, eosinophils, monocytes and neutrophils in the low and middle doses.

A significant reduction in thrombocytic parameters, such as platelet count and mean platelet volume indicates that the anti-thrombopoetin potency of the extract and the blood clotting mechanism of the animals will be inadequate with consequent effects of high loss of blood during injury (Lawal *et al*, 2015b) [18]. The 300 - 450 mg/kg extract showed a significant ($p < 0.05$ - $p < 0.01$) reduction in platelets and this effect was corroborated by a non-significant activity of the clotting time.

The liver filters and processes blood as it circulates through the body, metabolizes nutrients, detoxifies harmful substances and produces blood clotting proteins and bile among others. Damage to liver cells results in leakage of enzymes such aspartate aminotransferase (AST) and alanine aminotransferase (ALT) into the blood, while blood levels of alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) rise when bile flow is slow or blocked.

Serum aminotransferase levels—ALT and AST—are two of the most useful measures of liver parenchymal cell injury. AST is raised in acute liver damage, but is also present in RBCs, cardiac and skeletal muscle, so is not specific to the liver, while ALT is almost exclusively found in the liver (Nyblom *et al*, 2006) [19]. Elevated levels of ALT and AST indicate liver damage but are not good measures of liver function since they do not reliably reflect the synthetic ability of the liver and they may come from tissues other than liver such as muscle. The extract produced a non-significant increase in AST that could have resulted from extra-hepatic sources as well. There was a significant increase in ALT in the low and middle dose of the extract indicating mild hepatic damage. This corroborated the histologic findings of mild inflammation and could have resulted from the constituent of the extract menthofuran (Udobang, Bassey and Okokon, 2017) [6, 12] which is known to be hepatotoxic.

Biliary tract disease produces relatively greater increases in ALP than increases in ALT, AST, or lactose dehydrogenase (LD). ALP is associated with the plasma membrane of hepatocytes adjacent to the biliary canaliculus. Accordingly, diseases that predominately affect hepatocyte secretion due to obstruction or inflammation of the biliary tract will be accompanied by elevations of ALP levels. Bile-duct obstruction, primary sclerosing cholangitis, and primary biliary cirrhosis are some examples in which elevated ALP levels are often predominant over transaminase level elevation. Similar to ALT and AST, ALP is not specific for biliary tract disease since it is also released by osteoblasts, the ileum, and the placenta (Feldman, Friedman and Brandt, 2010).

Elevations of the AST level may also be seen in acute injury to cardiac or skeletal muscle or to a lesser degree after vigorous exercise. Diseases that primarily affect hepatocytes, such as viral hepatitis, will cause disproportionate elevations of the AST and ALT levels compared with ALP level. Common causes of mild increases in AST and ALT levels include non-alcoholic fatty liver disease, hepatitis C, alcoholic

fatty liver disease, and medication effect (e.g. due to statins). Total ALP elevation due to the hepatic fraction is a sensitive indicator of intra or extra hepatic cholestasis. Marked ALP elevation is usually noted with extra-hepatic biliary obstruction, primary biliary cirrhosis, and infiltrative processes such as neoplasm. Less dramatic ALP elevation can be seen in infectious mononucleosis, bile duct obstruction, hepatitis, heavy alcohol consumption and fatty liver. The significant increase in ALP in the middle dose of the extract could indicate the limited inflammation reported in the histology but not cholestasis.

Bilirubin is produced from the normal breakdown of pigment-containing proteins, such as hemoglobin from senescent RBC and myoglobin from muscle breakdown and may be elevated in hemolytic anemia. An elevated level of conjugated serum bilirubin implies liver disease. Also, only conjugated bilirubin appears in urine (unconjugated bilirubin is albumin bound and water insoluble). The presence of bilirubin in urine almost always implies liver disease. When the total bilirubin level is elevated and fractionation shows that the major portion (≥90 %) is unconjugated, liver disease is never the explanation, instead Gilbert disease or hemolysis is implied. (Murali and Carey, 2014) [21]. the extract induced no significant increase in total and conjugated bilirubin, indicating that the capacity of the liver to excrete bilirubin was not impaired.

The most sensitive marker of proteinuria is elevated urine albumin. Albumin, an essential protein made specifically by the liver is the main constituent of total protein and is decreased in conditions with severe chronic liver diseases such as cirrhosis and nephrotic syndrome, where it is lost through the urine leading to edema. Persistent presence of more than 30 mg albumin per gram creatinine in the urine is diagnostic of chronic kidney disease. There was no significant effect on the level of total protein and albumin, indicating that the protein synthetic ability of the liver was not altered. This is so because serum bilirubin is an index of hepatocytes ability to carry out synthetic function and does not change in mild liver injury but declines in cases of sub massive liver injury (Oyewole, Oladipupo and Atoyebi, 2012) [22].

The kidneys function to filter waste products of metabolism, such as urea from protein metabolism and uric acid from DNA breakdown from the body, regulate levels of electrolytes (eg. sodium, potassium and phosphate), acid-base balance of the body and blood pressure and produce erythropoietin which stimulates the bone marrow to produce red blood cells. The two waste products usually measured are blood urea nitrogen (BUN), and creatinine. Creatinine, a waste product of meat protein in the diet and also of the normal wear and tear on muscles is usually completely filtered from the blood by the kidneys (Oh, 2011) [23]. Normal Serum creatinine level range is 0.6 - 1.2 mg/dl and this can vary depending on age, race and body size. A creatinine level higher than 1.2 for women and 1.4 for men is a sign of a kidney problem. As kidney disease progresses, the level of creatinine in the blood increases. Urea is a breakdown product of food protein. A normal blood urea nitrogen (BUN) level is 7 - 20 mg/dl. As kidney function decreases, the BUN level increases. Common medications, including large doses of aspirin and some antibiotics, can also increase BUN, however BUN and creatinine will not be raised above the normal range until 60% of total kidney function is lost. Hence, the more accurate glomerular filtration rate (GFR) or its approximation of the creatinine clearance is measured whenever renal disease is suspected. (Pincus and Abraham, 2011) [24]. The extract produced no significant effect on the levels of $Na^+$, $CL^-$, $K^+$, $HCO^-_3$, and creatinine, but there was a significant ($p < 0.001$) increase of urea in the low dose of extract. The fact that there was no significant effect on creatinine, the most common indicator of renal injury that rises with marked damage to functioning nephrons and the rise in urea only in the low dose of the extract corroborates the negligible toxic effect seen on histology.

## 6. Conflict of interest statement

We declare that we have no conflict of interest.

## 7. Acknowledgments

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